UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE VETERINARIA

Departamento de Sanidad Animal

TESIS DOCTORAL

Identification and molecular characterization of bacteria isolated from human, animal, and food origins from the northern region of Ghana

Identificación y caracterización molecular de aislados de bacterias de origen humano, animal y alimentario en la región norte de Ghana

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Courage Kosi Setsoafia Saba

Director

Bruno González Zorn

Madrid, 2013

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Bajo la dirección del Profesor

Bruno González Zorn

Madrid, 2012

Don Bruno González Zorn, Profesor Titular del Departamento de Sanidad Animal de la Facultad de Veterinaria de la Universidad Complutense de Madrid (UCM),

CERTIFICA:

Que la tesis doctoral titulada "Identificación y caracterización molecular de aislados de bacterias de origen humano, animal y alimentario en la Región Norte de Ghana" que presenta el Licenciado en Ciencias en Tecnología de la Agricultura por la *University for Development Studies de Ghana* D. Courage Kosi Setsoafia Saba, ha sido realizada en las dependencias del Departamento de Sanidad Animal de la Facultad de Veterinaria de la Universidad Complutense de Madrid bajo mi dirección y cumple todas las condiciones exigidas para optar al grado de Doctor.

De acuerdo con la normativa vigente, firmo el presente certificado, autorizando su presentación como director de la mencionada tesis doctoral en Madrid a dieciocho de Septiembre de dos mil doce.

Firmado: 

“Everybody is a genius. But if you judge a fish by its ability to climb a tree, it will live its whole life believing that it is stupid.”
— Albert Einstein

I dedicate this Ph.D. Thesis to my parents……..

“Gratitude is the best attitude.”
— Anonymous

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“He who thus considers things in their first growth and origin ... will obtain the clearest view of them.”
— Aristotle

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List of abbreviations

AAFP: *Africa Association for Food Protection*

AFENET: *African Field Epidemiology Network*

AFIC: *Asian Food Information Centre*

APEC-FSCF: *Asian Pacific Economic Cooperation Food Safety Cooperation Forum*

AU: *African Union*

bp: *base pairs*

CCAFRICA: *Codex Coordinating Committee for Africa*

CLSI: Clinical Laboratory Standards Institute

CTX-M: *Cefotaxime Modifying enzyme*

DNA: *Deoxyribonucleic Acid*

EFSA: *European Food Safety Authority*

ESBLs: *Extended-spectrum beta lactamase*

et al.: *and others*

etc.: *et cetera (meaning "and other things")*

EU: *European Union*

FAO: *Food and Agriculture Organization*

FSANZ: *Food Standards Australia New Zealand*

FSIS: *Food Safety and Inspection Service*

FSLA: *Food Safety for Latin America*

GFDB: *Ghana Food and Drugs Board*

GFSI: *Global Food Safety Initiative*

GSB: *Ghana Standard Boards*

HACCP: *Hazard Analysis and Critical Control Point*

IAFP: *International Association for Food Protection*

IFPRI: *International Food Policy and Research*

ILSI: *International Life Sciences Institute*

IPPC: *International Plant Protection Convention*

ISO: *International Organization for Standardization*

kb: *kilobase*

MAFA: *Ministry of Agriculture and Forestry in New Zealand*

MIC: *Minimun Inhibitory concentration*

OECD: *Organization for Economic Co-operation and Development*

OIE: *The World Organization for Animal Health*

PAHO: *Pan America Health Organization*

PCR: *Polymerase Chain Reaction*

PFGE: *Pulsed Field Gel Electrophoresis*

QRA: *Qualitative Risk Analysis*

RNA: *Ribonucleic Acid*

SFI: *Safe Food International*

SPI: *Salmonella Pathogenicity Islands*

T3SS: *Type III secretion systems*

USDA: *United States Agricultural Department*

USFDB: *United States of America has United States Food and Drugs Boards*

WHO: *World Health Organization*

WTO: *World Trade Organization*

“For the things we have to learn before we can do, we learn by doing.”
—Aristotle

Resumen

Esta Tesis Doctoral está estructurada por artículos y consta de tres publicaciones, además de una introducción amplia sobre cada publicación y sobre la seguridad alimentaria a nivel mundial, centrándose luego en la situación de la misma en África y en Ghana en particular. Al final del trabajo de investigación se realiza una discusión global que aúna y complementa las discusiones de las publicaciones y se exponen las conclusiones que se generan de esta Tesis.

Situación Global de la Seguridad Alimentaria y de las Enfermedades Transmitidas por Alimentos

Actualmente, la seguridad alimentaria es una amenaza global que provoca una situación de riesgo que merece la atención, tanto de los países desarrollados como de los países en vías de desarrollo. Mientras los países desarrollados cuentan con reglamentos que regulan sus cadenas alimentarias, algunos países en vías de desarrollo (países africanos) todavía no tienen ningún reglamento que se ocupe de controlar el origen y la calidad sanitaria de los alimentos de consumo. Debido a la concienciación sobre la importancia de la seguridad alimentaria que está teniendo lugar a lo largo de todo el planeta, muchos países africanos (Ghana, Kenia, Sudáfrica, etc.) están promulgando leyes referentes a la seguridad alimentaria para proteger y controlar la salubridad y la calidad de los alimentos. Antes, los países en vías de desarrollo centraban su atención en solucionar el problema de la escasez de alimentos para paliar la hambruna que asolaba a la población, sin prestarle tanta atención a la calidad sanitaria de los alimentos, pero esto está cambiando para otorgarle a la seguridad alimentaria la atención que también requiere.

Por otra parte, hay que tener en cuenta que un gran numero de países de África carecen de autoridades que se ocupen de la seguridad alimentaria, como

por ejemplo la Autoridad Europea de Seguridad Alimentaria (EFSA), que se encarga de la seguridad alimentaria de todos los países europeos, además de los diferentes organismos que cada país tiene para regular y garantizar la seguridad de sus alimentos. La situación de la seguridad alimentaria en los países en vías de desarrollo ha mejorado notablemente, aunque lo hace a un ritmo muy lento. Por ejemplo, a titulo personal, he sido testigo de cómo en Ghana a principios de los 90, se ha pasado de utilizar hojas para envolver los alimentos a utilizar bolsas de plástico 10 años después. De la misma manera, ahora se utilizan bolsas de plástico de uso individual para servir el agua, en lugar de un solo vaso que era de uso común.

No obstante, algunas organizaciones internacionales como la Organización Mundial de la Salud (OMS), la Organización para la Alimentación y la Agricultura (FAO), el *Codex Alimentarius* etc., han jugado un papel muy importante en la seguridad alimentaria tanto al nivel mundial como en los países en vías de desarrollo, particularmente en África. De hecho, queda mucho por mejorar aun en los países de África y los gobiernos de estos países deberían colaborar con miembros del sector académico e industrial para poder mejorar la situación. Según estimaciones de la OMS, cerca de 2,2 millones de personas mueren cada año a causa de diarreas en el mundo, siendo 1,8 millones de los mismos niños (WHO 2008b). De los 1,8 millones de niños afectados, la mitad de estas muertes corresponde a niños africanos. Al realizar esta tesis hemos encontrado datos tan llamativos como que en 2010 en África murieron 596.050 personas de malaria, lo que corresponde al 91% de las muertes causadas por malaria a nivel mundial. Pero hay que tener en cuenta que según la OMS, más de 800,000 niños mueren de diarrea cada año en África, cifra mucho mayor que las muertes por malaria, como

se puede comprobar. No obstante, el fondo global dedicado a la prevención de la malaria alcanza mas de 2000 millones de dólares mientras que el fondo inicial destinado a la seguridad alimentaria empezó a funcionar en 2011 con una dotación de tan solo un millón de dólares. Los donantes de estos fondos tienen que ser conscientes de que las enfermedades transmitidas por alimentos causan más muertes que la malaria en África y platearse realizar las intervenciones necesarias en seguridad alimentaria.

Los estudios que se están realizando muestran que las enfermedades transmitidas por alimentos están aumentando de tal modo que la carga económica que suponen los pacientes para los gobiernos es cada vez mayor. Muchas de estas enfermedades se pueden prevenir, pero la mayoría de los países de África no cuentan con laboratorios adecuados para realizar análisis clínicos en caso de un brote o para realizar estudios de prevalencia de las enfermedades transmitidas por alimentos. De esta manera, actualmente, existe una imposibilidad para realizar una intervención orientada a controlar la situación y minimizar los daños.

Ghana y su Región Norte

Ghana es un país situado en la región occidental del África Subsahariana que cuenta con una población de 25 millones de habitantes. Los frecuentes conflictos políticos en los distintos países africanos representan un motivo de preocupación de alcance global. Sin embargo, en las últimas décadas Ghana ha logrado progresivamente su desarrollo económico, social y político y ha conseguido llevar a cabo elecciones de forma pacífica, así como suaves transiciones de poder. Estos logros han hecho que el resto de países africanos tomen a Ghana como un modelo de desarrollo. También ha servido de país de acogida a multitud de refugiados

provenientes de países vecinos en conflicto. Actualmente, el país acoge a miles de refugiados de Costa de Marfil y Liberia y el gobierno ghanés se responsabiliza de ellos, a pesar de la crisis económica.

Sin embargo, a pesar de esforzarse en ser un modelo a seguir para el resto del continente africano, no se pueden ignorar las amenazas y la pobreza que afectan a Ghana. Uno de sus puntos más débiles es la diferencia existente entre las regiones del norte y del sur del país. Su capital, Accra, que se encuentra localizada en el sur, ha sido, a lo largo de estos últimos años, el motor de desarrollo en todos los sectores de esta región, dando lugar a un desarrollo desigual con respecto al norte del país. El mayor reto para el gobierno es, precisamente, acabar con la enorme diferencia existente entre regiones.

Seguridad Alimentaria Microbiológica en Ghana: Un Meta-Análisis

En la primera publicación que presentamos en esta tesis doctoral hemos realizado un meta-análisis de los artículos de PubMed sobre seguridad alimentaria microbiológica en Ghana. Hemos evaluado estos artículos basándonos en las localizaciones de las investigaciones, las bacterias identificadas en los artículos, los tipos de alimentos, los procesos de elaboración de los alimentos antes la toma de muestras y otros criterios para juzgar el rigor científico de las investigaciones.

Hemos constatado que hay pocas publicaciones sobre seguridad alimentaria en Ghana y que casi todas las publicaciones hacen referencia a la Región Sur, por lo cual no hay datos suficientes que nos permitan conocer la situación general en la Región Norte de Ghana. Hemos visto que las bacterias aisladas con mayor frequencia en los alimentos fueron *Enterobacter spp.*, *Citrobacter spp., Klebsiella spp.* y *Escherichia spp.,* en porcentajes del 65%, 50%,

46% y 38% respectivamente. La mayoría de los artículos centran sus investigaciones en una única especie bacteriana, y tres de ellos no mencionan los agentes etiológicos, al ser artículos generales sobre seguridad alimentaria microbiológica. En la mayoría de los artículos las muestras consistían en alimentos ya procesados, pero es muy importante incluir en las investigaciones todos los procesos que se aplican a los alimentos desde el campo hasta el momento de su consumo, controlando también la calidad sanitaria de las materias primas. Esta metodología consistente en investigar todos los pasos y procesos por los que pasan los alimentos es un estándar extendido especialmente en los países avanzados para mantener la seguridad de la cadena alimentaria. También hemos observado que no hay ninguna publicación sobre pescado, alimento prioritario al que es muy importante prestarle atención, ya que constituye la fuente de proteínas básica para gran parte de la población en Ghana.

Sorprendentemente, sólo en uno de los artículos se realizan pruebas de susceptibilidad a antimicrobianos en los microorganismos aislados. Este tipo de estudios es muy importante para conocer la situación de las resistencias a antimicrobianos, ya que las resistencias constituyen una amenaza mundial. En todos los artículos usaron métodos clásicos de aislamiento e identificación bacteriana en lugar de técnicas moleculares de alta sensibilidad y especificidad como por ejemplo la PCR. La mayoría de las bacterias no fueron identificadas hasta el nivel de especie o serotipo, pero tener este dato es muy importante a la hora de realizar una intervención, ya que diferentes especies del mismo género pueden tener diferentes características que condicionan las medidas a tomar.

Primera Identificación de *Salmonella* Urbana y *Salmonella* Ouakam en Humanos en África

Para establecer la prevalencia de *Salmonella* en la región Norte de Ghana, en nuestra segunda publicación hemos llevado a acabo una investigación para aislar y identificar *Salmonella* en heces de pacientes de un hospital universitario de la región. Esta región es una de las zonas más pobres de Ghana, tanto en sus condiciones higiénico-sanitarias como en su infraestructura. La mayoría de las personas de las áreas rurales no tienen acceso al agua potable y toman agua de riachuelos y de ciertos refugios que cuentan con una fuente de agua. Estos riachuelos y refugios también son utilizados por animales, lo que supone una situación de riesgo evidente, ya que algunos animales son reservorios naturales de *Salmonella.* Además, no hay muchos estudios sobre *Salmonella* en la región que nos permitan conocer la situación real, por lo que un estudio sobre el tema resultaba muy conveniente.

Hemos tomado 101 muestras de heces de pacientes del hospital, la mayoría de los cuales presentaban diarrea. De las 101 muestras, cuatro fueron positivas para *Salmonella.* Hemos identificado los serotipos por el método de aglutinación. Los casos dudosos se identificaron por PCR. Hemos identificados *S.* Urbana, *S.* Ouakam, *S.* Stanleyville y *S.* Senftenberg. Los serotipos *S.* Stanleyville y *S.* Senftenberg habían sido identificados anteriormente en algunos brotes en algunas zonas de África y concretamente en Ghana, pero es la primera vez que se aislan en la Región Norte de Ghana. El serotipo *S.* Ouakam había sido aislado una vez en el norte de África, pero de alimentos, de modo que nuestro trabajo es el primero en el que se aisla en África de una muestra de origen humano. El serotipo *S.* Urbana nunca antes había sido aislado de humanos en África, por lo que nuestro

trabajo es también el primero en hacerlo. Este serotipo es frecuente en los reptiles, tanto domésticos como silvestres, y ha sido implicado en algunos brotes fatales. Sorprendentemente, ninguno de los serotipos aislados fue resistente a ninguno de los 20 antimicrobianos frente a los que se realizaron antibiogramas. Sospechamos que estos serotipos pueden proceder de reptiles u otros animales que han contaminado el agua de riachuelos y refugios, agua que posteriormente es utilizada por las personas para cocinar, beber y realizar otras actividades de la vida doméstica. Hemos sugerido realizar análisis del agua de los riachuelos y de los refugios y tomar más muestras de personas de la Región Norte de Ghana para recabar más información y poder realizar la intervención adecuada.

Identificación, Caracterización Molecular y Diversidad Genética de *Escherichia coli* de aislados procedentes de Humanos, Animales y Alimentos de la Región Norte de Ghana, West África

El centro del trabajo fue la caracterización molecular de aislados de *E. coli* procedentes de humanos, animales y alimentos. *E. coli* es una bacteria que forma parte de la microbiota intestinal de humanos y animales, es decir, es un microorganismo comensal que habita en el intestino de los animales homeotermos. En seguridad alimentaria *E. coli* es un indicador de contaminación fecal de los alimentos y de las aguas.

Por ello, hemos estudiado aislados de *E. coli* de pacientes de un hospital universitario, de gallinas ponedoras y de alimentos procedentes de la venta ambulante en la Región Norte de Ghana para conocer la situación de la seguridad alimentaria en esta área, el perfil de resistencia a antimicrobianos de la bacteria y los mecanismos de resistencia a antimicrobianos que presenta. Hemos tomado 101 muestras fecales de humanos, de los que en 99 se aisló *E. coli*. Hemos

recogido también 30 muestras fecales de cinco granjas avícolas diferentes, aislándose *E. coli* en todas las muestras. Hemos tomado 49 muestras de alimentos de puestos de comida ambulantes, de las que 15 fueron positivas a *E. coli*.

Los resultados de las muestras tomadas de alimentos demuestran la existencia de contaminación fecal, ya que 15 eran positivos a *E. coli.* Para conocer el perfil de susceptibilidad a antimicrobianos se realizaron antibiogramas en los que se utilizaron 20 antibióticos diferentes. Ninguno de los aislados de los diferentes orígenes resultó resistente a amikacina, apramicina, colistina o imipenem por lo que estos antibióticos podrían ser los de elección en la Región Norte de Ghana para tratar enfermedades infecciosas causadas por bacterias resistentes a otros antibióticos de uso común.

La resistencia a antimicrobianos de los aislados procedentes de las granjas de aves ponedoras era mucho mayor que la de los aislados de las granjas de pollos dedicados al consumo de carne. Las gallinas ponedoras tienen un ciclo productivo de algo mas de un año, mientras que los pollos de carne están algo mas de dos meses en las granjas. Por tanto, la presión antibiótica a la que está sometida la microbiota de las gallinas ponedoras es mayor que la de los pollos de carne, lo que puede explicar la mayor resistencia de los aislados de gallinas ponedoras. También hay que destacar el peligro que supone el uso de antibióticos como promotores del crecimiento en producción animal, ya que puede conllevar en la emergencia de resistencias que pueden afectar a los personas.

Aparición de la resistencia a cefalosporinas de tercera generación y otros genes de resistencia antibiótica en la Región Norte de Ghana

En un estudio anterior al nuestro sobre resistencia a antibióticos en Ghana publicado en 2008 (Djie-Maletz *et al.,* 2008), no se encontraron en *E. coli* resistencias a cefalosporinas de tercera generación. Sin embargo en nuestro estudio hemos identificado aislados de *E. coli* de origen humano resistentes a cefalosporinas de tercera generación. Eso significa que en los próximos años se tendrá que optar por antibióticos más potentes y caros para el tratamiento de procesos infecciosos. Pero hay que tener en cuenta que esta población es la de menor poder adquisitivo de Ghana y la situación puede ser por ello, aun más grave. Con la aparición de la resistencia a cefalosporinas de tercera generación, las autoridades de los hospitales deberían establecer una política general sobre el uso de los antibióticos en el ámbito hospitalario con el fin de controlo. Además, las autoridades sanitarias deberían promover el uso responsable de los antibióticos entre la población para evitar la automedicación, fenómeno frecuente en Ghana.

Además, los dos aislados (BB1094 y BB1095) resistentes a cefalosporinas presentan otros genes de resistencia frente a otros antibióticos, de tal modo que BB1094 fue resistente a nueve antibióticos y BB1095 a once antibióticos. Las dos cepas tienen la enzima TEM-1, una betalactamasa que confiere un alto nivel de resistencia a ampicilina. Ambas cepas son también productoras de enzimas que reciben el nombre de betalactamasas de espectro extendido (ESBLs). El tipo de ESBL que presentan es CTX-M-15, considerada pandémica. La cepa de *E. coli* patogénica que causó la muerte a más de 50 personas en Alemania en 2011 producía también esta enzima (Januszkiewicz *et al.,* 2012). Además, la cepa BB1095 tiene un plásmido de 97 kb. Mediante técnicas moleculares hemos

observado que este plásmido codifica un *qnrS1* que disminuye la susceptibilidad de las bacterias a la ciprofloxacina. Las dos cepas tienen una integrasa de clase 1. Mientras la cepa BB1095 no presenta ningún casete de genes en el mismo, el integrasa de BB1094 tiene un casete con un gen, *dfrA*1, que confiere resistencia a trimetoprim.

Determinación del Grupo Filogenético y Electroforesis en Gel de Campo Pulsado

E. coli se puede clasificar en diferentes grupos filogenéticos: A, B1, B2 y D. Las cepas que presentan más factores de virulencia y que son responsables de infecciones extra-intestinales provienen de los grupos B2 o D, mientras que los comensales suelen pertenecer a los grupos A o B1. Hemos realizado una PCR triple para determinar el grupo filogenético de todas las cepas de origen humano, animal y alimentario (Clermont *et al.,* 2000). Hemos obtenido los siguientes resultados en los aislados de origen humano: grupo A, 41 cepas (46%); grupo B1, 29 cepas (33%); grupo B2, 7cepas (8%); y grupo D, 12 cepas (13%). Las cepas de las granjas avícolas se dividieron en grupo A, 16 cepas (53%); grupo B1, 10 cepas (33%); grupo B2, 1 cepa (3%) y grupo D 3 cepas (10%). En los aislados de origen alimentario solo había cepas de dos grupos filogenéticos diferentes, del grupo A, 13 cepas(87%) y del grupo B1, 2 cepas.

La mayoría de las cepas aisladas de los diferentes orígenes pertenecen al grupo A, lo que significa que la mayoría de las cepas de *E. coli* aisladas son de tipo comensal más que patógeno. Pero algunos estudios recientes muestran que las cepas de los grupos A y B1 también pueden inducir factores de virulencia presentes en las cepas patógenas. Entre las cepas procedentes de humanos y animales, había 7 cepas de origen humano y 1 cepa de origen animal que

pertenecen al grupo B2, que es el grupo que aglutina las cepas más virulentas. La aparición de estas cepas patógenas representa un peligro para los hospitales y para la salud pública de la Región y de todo el país.

Hemos estudiado la proximidad genética de todas las cepas mediante Electroforesis en Gel de Campo Pulsado (PFGE). Hemos observado que algunas cepas de diferentes orígenes están relacionadas genéticamente. Este resultado es muy importante ya que puede implicar un flujo de cepas de *E. coli* que se encuentran circulando entre humanos, animales y alimentos. Estos resultados pueden ayudar a las autoridades sanitarias de la Región Norte de Ghana a controlar este flujo de cepas de *E. coli* patógenas entre humanos, animales y alimentos.

Esta Tesis Doctoral representa un papel muy importante tanto en la Región Norte de Ghana, como en todo el país, ya que es la primera vez que se realiza un trabajo de investigación de esta naturaleza en Ghana. Además, esta Tesis tiene como objetivo principal ser el punto de partida para futuros estudios orientados a mejorar la situación de la seguridad alimentaria en Ghana.

“The fear of failure helps you to prepare adequately to prove your enemies wrong, but excessive fear without courage leads you to failure."
—Courage Saba

Introduction

1.0 Food Safety and Foodborne Illnesses

1.1 General and Brief Overview of Food Safety and Foodborne Illnesses Worldwide

The definition of Food Safety is complex as it involves a combination of multifaceted processes that are interdisciplinary. Food safety can be generally defined as the science and art of producing, harvesting, processing, handling, packaging, storing (freezing), transporting and distributing or selling food in an acceptable manner (by standards) that will prevent the food from changing its biological/microbiological, chemical and physical form beyond acceptable limits until it reaches the last consumer for consumption. This necessitates a holistic approach to food safety from farm to fork and hence makes it a multidisciplinary subject. What may be an accepted standard in one country may not be acceptable in another country as each country has its standard apart from the internationally accepted one to protect its citizens. Most developing countries, however, tend to adopt standards from developed countries, since their standards are seen as more advanced (Stepheson, 1997). This phenomenon forces most developing countries adopt foreign food safety policies to meet standards that will improve the acceptability of their export products rather than setting food standards suitable for their local conditions. These standards adopted by African countries sometimes generate a conflict of interest where the institutions, agencies or authorities working to regulate and control food are not under one umbrella. A typical scenario of conflict of interest between food or standards regulatory bodies is the Ghanaian situation where there are at times conflicts of interest between the Ghana Food and Drugs Board and the Ghana Standards Board.

Food safety issues have been given critical attentions especially in the developed countries for the past decades. However, food safety, until recently has not attracted the attention of most governments in developing countries especially Africa. Governments from most developed countries have enacted laws to regulate food and prevent its citizens from being exposed to the consumption of unsafe food. However, food safety issues such as regulations, laws enactment or enforcement or scientific based analysis in developing or resource-limited (Africa) countries have had a slow development in the past decades even though now it is increasing. In recent times, most African countries (e.g. Ghana, South Africa, Kenya etc.) are strengthening their food safety regulations to protect their citizens and meet international standards. A typical example of change I have observed in Ghana during my childhood from the 1990s to now was the change in the use of broad tree leaves (with minimal cleaning) to package ready-to-eat food to the use of polythene bags. Another example is the change in the use of the same cup to serve commercially sold water to various customers to the use of polythene bags to serve water, commonly called 'pure water', which are sold to one person only. Even though there may be criticism about the safety of those packaging bags, I dare say it is less risky than serving food in broad leaves, which have been exposed to the environment and accumulate dust, droppings of birds, lizards and other insects. Due to an increased response to an increasing number of food safety problems and rising consumer concerns, many governments, institutions, and non-governmental organizations are keen on food safety issues (WHO, 2007a). Although food safety cuts across all the spheres of food, one important realm of concern especially in the developing countries is the safety of street foods. This may be due to the increasing number of foodborne diseases as result of inadequate knowledge on food safety

issues (Rane, 2011). Street food is ready-to-eat food or drink sold in the street or other public places, such as market or fairs, by a hawker or vendor often from a portable stall (Simopoulos, 2000). About 2.5 billion people consume street food every day worldwide (FAO, 2007a) and most of these people are found in developing countries, especially Africa, since they are relatively cheap and more affordable for the less income groups.

The first international standards commission that sought to tackle the issue of food quality and safety globally and holistically was the Codex Alimentarius Commission, which was established by FAO and WHO in 1963 to streamline food standards worldwide (Codex Alimentus, 1963). Other organizations and related associations that seek to promote food safety and quality worldwide are the WTO, ISO, IPPC, OIE, IFPRI, OECD, ILSI, IAFP, SFI, GFSI, etc. There are also continental and regional authorities/associations/organizations/boards that seek to promote and regulate food safety in their respective areas. The United States of America has the USFDB and FSIS under the USDA. The European Union has the EFSA. In Asia, the Asia FoodNet under the WHO plays a very important role in food safety and foodborne illnesses. There are other organizations/centers that aid to promote food safety in the Asian continent, for instance, the Asian Food Information Centre (AFIC) and the Asian Pacific Economic Cooperation Food Safety Cooperation Forum (APEC-FSCF). Australia and New Zealand have a joint authority called the Food Standards Australia New Zealand (FSANZ). There is no notable agency, association or authority that addresses food safety issues in the African continent apart from WHO, FAO and the Codex Coordinating Committee for Africa (CCAFRICA) but there are newly formed associations called Africa Association for Food Protection (AAFP) and the African Field Epidemiology Network (AFENET), that seek to link food

safety issues and foodborne illnesses in all the African countries. The Pan America Health Organization (PAHO) is the main body that promotes awareness on foodborne diseases and food safety in the Latin American countries. There is also a nascent organization called Food Safety for Latin America (FSLA), which promotes food safety in that continent. The WHO, FAO and Codex Alimentarius work in all the continents to ensure food safety and prevent foodborne illnesses especially in its member states. The WHO/FAO effort in promoting food safety among its members was strengthened by the formation of the International Food Safety authorities Network (INFOSAN) in 2004 to promote the rapid exchange of information during food safety related events among member states (INFOSAN Report, 2011).

According to WHO (2007a), "foodborne illnesses are defined as diseases, usually, either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food". It has been estimated that 2.2 million deaths can be attributed to diarrheal diseases yearly, of which 1.8 million occur in children less than 5 yrs. of age (WHO, 2008b). There is little information of surveillance data on foodborne diseases in Africa, Asia, Latin America and Caribbean regions and information gathered is from specific but limited investigations and studies, (Todd, 1997, Pires *et al*., 2012). Table 1 illustrates the estimated occurrence of bacterial infections and intoxication from selected regions. Recorded data from developing countries seems to be outdated due to lack of continuous research data (Henson, 2003). However, a lot of scientific researches as well as surveillance are carried out in Europe and North America and hence are able observe the trend of foodborne diseases and implement effective control measures to curb them.

Table 1. Estimated occurrence of bacterial infections and intoxication in selected regions.

Disease	Africa	Central & South America	South East Asia	Western Pacific
Bacillus cereus gastroenteritics	+++	+++	+++	+++
Botulism	+	+	+	+
Brucellosis	+/++	++	+/++	+/++
Campylobacteriosis	+++	+++	+++	+++
Cholera	+/++	+/++	+	+
Clostridium perfringens enteritis	+++	+++	+++	+++
Escherichia coli disease	+++	+++	+++	+++
Listeriosis	+	+	+	+
Typhoid and paratyphoid fever	++	++	++	++
Salmonellosis	+++	+++	+++	+++
Shigellosis	+++	+++	+++	+++
Staphylococcus aureus intoxication	+++	+++	+++	+++
Vibrio parahaemolyticus enteritis			++	++
Vibrio vulnificus septicaemia				++

Note: -: absent; +: occasional or rare; ++: frequent; +++: very frequent.
Source: WHO cited by Henson, 2003.

1.1.1 Food safety and foodborne illnesses in the United States of America and Latin America

1.1.1.1 United States of America

The food safety culture and awareness in the United States of America (USA) is so advanced that, there is almost always daily news about the safety of foods. Thus, media attention related to safety of food has heightened consumer awareness. The Food Safety and Inspection Service under the United States Department of Agriculture has a myriad of food safety education tips for US citizens which are freely accessible on their website (http://www.fsis.usda.gov/). Even though the country has a very well organized food safety programs compared to other countries, there is still a problem of unsafe food consumption and foodborne illnesses are recorded frequently**.** The free accessibility of food safety data to the public is a critical point in the risk communication process in the US. This has helped the US citizens to be abreast of food safety issues as well

as foodborne illnesses and take precautionary measures to reduce the scourge. The USA is also a member of the APEC-Food Safety Cooperation Forum. The forum was established in 2007 to promote food safety among member countries. The Food Safety Cooperation Forum's Partnership Training Institute Network (FSCF PTIN) was created to harmonize the issues of food safety within member countries (http://fscf-ptin.apec.org).

Recent reports from the US showed an increase in episodes of foodborne illnesses with 55,961 hospitalization and 1,351 deaths, but while most illnesses were caused by norovirus, non-typhoid Salmonella remains the leading cause of death (Scallan *et al.*, 2011a). Figure 1 below shows a schematic diagram of estimated of illnesses, hospitalizations, and deaths caused by unspecified acute gastroenteritis agents. Interestingly, the most scaring data about unspecified agents involved in foodborne diseases is that, they might have caused more hospitalizations and deaths than specific agents implicated (Scallan *et al.*, 2011b).

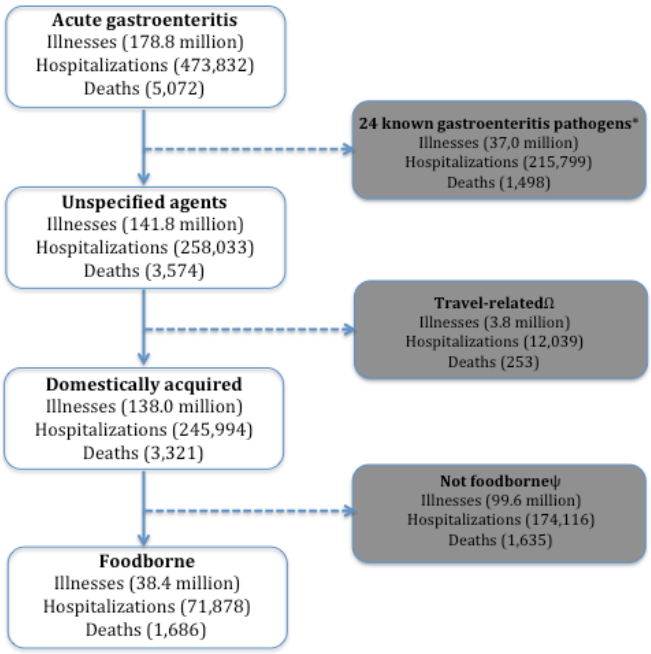

Figure 1. Schematic estimates of illnesses, hospitalizations, and deaths caused by unspecific acute gastroenteritis agents. *The estimated numbers of illnesses, hospitalizations, and deaths (hereafter, illnesses refers to illnesses, hospitalizations, or deaths as appropriate) caused by 24 known gastroenteritis pathogens [15] were subtracted to estimate the number of illnesses caused by unspecified agents. Ω The estimated number of illnesses related to travel were subtracted to estimate the number of domestically acquired illnesses. The estimates of the proportion related travels were based on the overall weighted distribution of the proportions of illnesses that were related to travel from the 24 known gastroenteritis pathogens. Ψ The estimated number of nonfoodborne illnesses were subtracted to estimate foodborne illness. The estimates of the proportion foodborne were based on the overall weighted distribution of the proportions of illnesses that were foodborne from the 24 known gastroenteritis pathogens. All estimates were based on US population in 2006.
Source: Scallan et al., 2011b.

1.1.1.2 Latin America

Unlike the United States of America and Europe, Latin American countries do not have a common effective and efficient body that co-ordinates and regulates food safety issues. However, there are national authorities and boards that regulate food safety in their respective countries. The PAHO is the main body that promotes awareness on foodborne diseases and food safety among Latin American countries; this organization has an institute called *El Instituto Panamericano de Protección de Alimentos y Zoonosis* (PANALIMENTOS), which is

specifically focuses on food safety and foodborne illness surveillance (http://www.panalimentos.org). There is an organization called Food Safety for Latin America (FSLA) that is now seeking to link all the Latin American countries to promote food safety in the continent. However, there may be difficulties in communicating research findings and outbreaks of foodborne illnesses to most of the people living in this continent because of inaccessibility to the internet unlike the US or Europe populace who have easy access to the internet.

In a recent investigation carried out on foodborne pathogens, outbreaks and surveillance in the continent, there have been reports of foodborne illness outbreaks mainly caused by bacteria reported from some countries who usually have some intermittent data mostly implicating meat, dairy products, water and vegetables were mostly implicated (Pires *et al.*, 2012). However, the level of cooperation to collect and report on foodborne diseases within and among the Latin American and Caribbean countries seem to be stronger than that of African countries. Table 2 includes the various countries and pathogens involved in the investigation.

In recent times, at country levels there have been reports of virulent foodborne pathogens. For instance, the first report of the presence of *mecA* genes in *Staphylococcus aureus* isolated from ready-to-eat food in Brazil and Latin America (Rizek *et al*., 2011) and the first molecular *E. coli* report confirming the presence of *E. coli* pathotypes circulating in Colombia among children with diarrhea and food products for human consumption (Rugeles *et al*., 2010).

Table 2. Number of outbreaks by pathogens reported by each country in the Latin America and Caribbean countries (1993-2010)

Etiology / Country	*B. cereus*	*C. perfringens*	*E. Coli*	*S. Aureus*	*Salmonella*	*Shigella* spp.	*V. Cholerae*	*V. Parahemolyticus*	Others[a]	*Total*
Argentina	13	20	1				1		0	35
Bahamas	7	6							13	26
Barbados								1	1	
Bolivia					5				0	5
Brazil		25							0	25
Chile	14	125	182	64	139	105		513	2632	3781
Colombia	1								0	1
Costa Rica	1	18	7	5	20	65			0	116
Cuba		3		852	761	122	23	9	43	1813
Ecuador		5	2				2		1	10
El Salvador	7	3						2	12	
Guatemala	9							0	9	
Mexico	9		144						15	168
Nicaragua	1	35	12	19	4		15		8	103
Panama					2				0	2
Paraguay	1	2	3	6	15	3	3		12	45
Peru		1							2	8
Dominican Rep	4	7	11						2	24
Uruguay	8	2	2						21	33
Venezuela	10	30	26						30	96
Total	69	295	402	946	946	294	44	522	2776	6313

[a] Includes unknown
Source: Pires et al., 2012.

1.1.2 Food safety and foodborne illnesses in Europe, Australia, New Zealand and Asia

1.1.2.1 Europe

The authority responsible for Food Safety in the Europe Union (EU) is the European Food Safety Authority (EFSA). The European Centre for Disease Prevention and Control (ECDC) collaborates with EFSA in the detection and prevention of foodborne illnesses. Reports from EFSA and ECDC showed an increasing trend in foodborne outbreaks and illnesses apart from a few successes that have been chalked up in the reduction of salmonellosis among the member states, but most of the 5,262 reported foodborne outbreaks in 2010 were caused by *Salmonella,* viruses, *Campylobacter and* bacterial toxins, and the main sources were eggs, mixed or buffet meals and vegetables (EFSA, 2012).

Table 3 shows a breakdown of the number of cases, hospitalized and deaths in the various countries in the EU. The EFSA has well-organized and controlled prevention and surveillance programs directed to reduce the incidence of foodborne illnesses and promote food safety in the member countries. Collaboration among member countries is highly attributed to tracking and curbing the 2011 *E. coli* outbreak in Germany and hence provided rapid alert needed to stop the spread of the deadly strain. Moreover, there are a lot of EU sponsored projects that are directed to investigate and give scientific evidence as to how foodborne illnesses can be curbed within and among the member states. The member states also have governmental and non-governmental bodies that promote food safety issues in their respective countries. However, there is limited cooperation within and among the African Union countries as well as Asia and Latin American continents to collate and report foodborne illnesses.

Table 3. Number of human cases in foodborne outbreaks (weak and strong evidence-excluding strong evidence waterborne outbreaks) in EU, 2010

Country	Strong evidence outbreaks				Weak evidence outbreaks			
		Human cases				Human cases		
	N	Cases	Hospitalized	Deaths	N	Cases	Hospitalized	Deaths
Austria	10	317	48	1	183	521	107	1
Belgium	16	651	45	0	89	543	15	0
Czech Rep.					25	807	42	0
Denmark	48	1,485	7	0	28	743	4	0
Estonia	2	105	7	0	30	215	31	0
Finland	24	562	1	0	19	361	6	0
France	75	1,407	224	1	964	8,561	466	0
Germany	40	500	66	2	399	1,878	273	1
Greece					3	193	48	0
Hungary	30	932	61	0	269	1,731	387	2
Ireland	3	43	19	0	10	55	10	0
Italy					225	1,205		
Latvia	7	77		0	498	1,438	2	0
Lithuania	7	83	54	0	141	402	300	0
Malta					50	166	3	0
Netherlands	13	213	63	2	238	1,001	12	1
Poland	118	1,407	354	1	333	4,709	752	0
Portugal	4	56	0	0	0			
Romania	19	326	95	1	10	143	119	0
Slovakia	20	262	65	0	467	2,405	513	0
Slovenia	3	121	0	0	0			
Spain	196	2,474	225	2	286	1,551	153	5
Sweden	13	292	12	0	280	2,078	20	0
U. K	50	1,093	76	5	17	358	10	0
EU Total	698	12,409	1,422	15	4,564	31,064	3,273	10
Norway	4	242	0	0	49	547	8	0
Switzerland	6	52	42	0	5	54	1	1

Source: EFSA, 2012

1.1.2.2 Australia and New Zealand

The authority that is responsible for food safety in Australia and New Zealand is the Food Standards Australia New Zealand (FSANZ). Just like the USA and the EU, the FSANZ has a well-organized educational program about food safety to educate their populace and to control foodborne illness outbreak and related illness in their regions (FSANZ, 2003). They however, have some regulations (chapters) that apply to either Australia or New Zealand only. The Ministry of Agriculture and Forestry in New Zealand is responsible for food regulations that are specific in its territory only (MAFA, 2011). The OzFoodNet is

responsible for foodborne illnesses surveillance in Australia and collaborates with the WHO to report survey foodborne illnesses (http://www.ozfoodnet.gov.au).

The recent surveillance data of the OzfoodNet revealed a large number of people who were affected by diseases transmitted by food (OzFoodNet, 2010). The report Identified *Campylobacter* as the most frequent cause of infections followed by *Salmonella* and also, more than 1,820 outbreaks of gastrointestinal illness that affected 36,421 individuals and resulted in 1,240 hospitalizations and 188 deaths. However, reports from the Ministry of Agriculture and Forestry in New Zealand indicated a significant reduction in foodborne infections caused by *Campylobacter* spp., *Salmonella* spp. and *Listeria* spp. since 2007 (MAFA, 2011) (Table 4). Australia and New Zealand are also part of the APEC-Food Safety Cooperation Forum.

Table 4. Reduction in foodborne diseases in New Zealand from 2007-2010

Foodborne Pathogen	Public Health Goal	2007 Baseline per 100, 00 population	2010 Observation	Percentage Reduction Achieved
Campylobacteriosis	50% reduction below the baseline	161.1	90.6 (7346 cases)	44
Salmonellosis	30% reduction below the baseline	14.2	12.8	10
Listeriosis	No increase in the annual foodborne rate	0.47	0.41 (18)	12.8

Source: Annual Report Concerning Foodborne Diseases in New Zealand 2010

1.1.2.3 Asia

The Asia FoodNet under the WHO plays a very important role in food safety and foodborne illnesses in Asia. In the Asian continent, the notable centers/organizations that are engaged in food safety issues in most of the countries are the AFIC and the APEC-FSCF. Unlike the APEC-FSCF, that is only

targetted at its member countries in the Asian region, the Asia FoodNet and AFIC seek to cover all the Asian countries. The Asian continent does not have a very comprehensive and efficient system for reporting foodborne diseases compared to the EFSA or USFDA. However, the individual countries in the continent have own their food authorities, agencies or boards that regulate food and control foodborne illnesses.

A number of foodborne illness outbreaks have been recorded in Asia in recent times. Chen *et al.* (2010) reported that foodborne illnesses are a major public concern in China. In their surveillance data analyzed from an outbreak in 2006, they reported 594 outbreaks of foodborne disease reported from 18 provinces, which affected 13,849 persons and killed 67 persons. They observed that microbes were implicated in the majority of cases. Other foodborne illness outbreaks or cases related to foodborne illness were recorded in China (Tang *et al*., 2010; Wen *et al*., 2011; Shao *et al.*, 2011; Alcorn *et al*., 2012), Japan (Mizoguchi *et al*., 2011), Taiwan (Su *et al*., 2005), India (Chaundhry *et al*. 1998; Bhunia *et al.,* 2009), Thailand (Woodring *et al.,* 2012), Malaysia (Meftanhunddin *et al*., 2012), and the food safety and public health concerns of the avian influenza in the Asian continent (Chmielewski *et al*., 2011).

1.1.3 Food safety and foodborne illnesses, and street foods in Africa

1.1.3.1 Food safety and foodborne illnesses

Apart from WHO, FAO, and the CCAFRICA, there is no notable effective and reliable authority, board, organization or association that seems to ensure food safety holistically in African countries. With the growing concern of food safety issues worldwide, most African governments are developing or had developed food safety policies. The political and economic situations in some of the regions

do not favor the implementation of effective food safety policies to help track down the issues of foodborne illnesses in the continent as well as preventing the spread to other continents (FAO/WHO, 2005a). There are however, some nascent associations: Africa Association for Food Protection and African Field Epidemiology Network who aim to promote food safety to curb frequent but silent foodborne illness outbreaks in the continent.

There have been improvements in the collection of foodborne illness or outbreak data in some developing continents such as Latin American and Caribbean, but such attempts are far from the reality in the African continent (Kaferstein, 2003). The lack of sufficient and continuous data from African countries could be justified by the fact that, as of the end of 2007, only Cameroon, Ethiopia, Madagascar, Nigeria, Senegal and South Africa reported data to the Global *Salmonella* Surveillance (Global Salm-Surv), a global network of laboratories and individuals involved in surveillance, isolation, identification and antimicrobial resistance testing of *Salmonella* and other food pathogens (WHO, 2007b). It is, therefore, not surprising that estimates of diseases from African countries are based on the little information that is obtained from few countries. Table 5 shows the number of some major scientifically published foodborne outbreaks from Africa on PubMed from 1969-2011.

Table 5. Evidence-based foodborne outbreaks compiled from PubMed from 1969-2011

Country	Organism/Chemical implicated	Reference
South Africa	Potassium bromate	Stewart *et al*. 1969
South Africa	Mussel poisoning	Grindley *et al*. 1969
Togo	*Vibrio parahaemolyticus*	Bockemuhl *et al*. 1972
Ghana	Alkyl-mercury fungicide	Derban, 1974
South Africa	*Vibrio parahaemolyticus*	Bubb, 1975
Central Africa	*Shigella dysenteriae*	Ebright *et al*., 1984
Rwanda	*Shigella dysenteriae*	Vimont-Vicary *et al*., 1985
Tanzania	Atopine-like alkaloid	Rwiza, 1991
Djibouti	Group A *Streptococcus*	Bercion *et al*., 1991
Egypt	*Clostridium botulinum*	Werber *et al*., 1993
DR Congo	Various	Milleliri *et al*., 1995
Burundi	*Shigella dysenteriae*	Ries *et al*., 1994
Malawi	*Vibro cholera*	Swerdlow *et al*., 1997
South Africa	*Shigella flexneri*	Karas *et al*., 2001
Swaziland	*E. coli* O157:H7	Effler *et al*, 2001
South Africa	*Clostridium botulinum*	Frean *et al*., 2004
Zambia	*Vibro cholera*	CDC, 2004a
Kenya	Aflatoxin	CDC, 2004b
Cameroon	*Plesiomonas shigelloides*	Wouafo *et al*., 2006
Egypt	Hepatitis A	Frank *et al*., 2007
South Africa	*Salmonella*	Smith *et al*., 2007
Central African Rep.	Konzo diseases	Mbelesso *et al*., 2009
Democratic Rep. Congo	*Salmonella*	Muyembe-Tamfum *et al*, 2009
Cameroon	Konzo dieasese	Ciglenečki *et al*., 2011
South Africa	*Salmonella*	Niehaus *et al*., 2011

Even though there may be a lot of foodborne outbreaks in Africa, only those countries with well-trained personnel and laboratories are capable of surveying and reporting foodborne outbreaks that are scientifically based. It is observed that South Africa had the highest number of reported cases because it has more laboratories that are capable of investigating outbreaks and incidences once there suspected cases. This result does not suggest that South Africa has the highest number of outbreak cases among the African countries. The pathogens that were found implicated in three outbreaks were *Shigella dysentriae* and *Salmonella*. *Vibrio parahaemolyticus*, *Vibrio cholera* and *Clostridium botulinum* were

implicated in two outbreaks. Pathogens implicated in a single outbreak were *E. coli O157*, *Shigella flexneri*, Group A *Streptococcus* and *Plesiomonas shigelloides.* The only viral outbreak was caused by Hepatitis A. Mussel was the only seafood implicated and potassium bromate, alkyl mercury fungicide and atopine-like alkanoid were the 3 chemical agents implicated in the outbreaks. Konzo diseases associated with cassava caused 2 of the outbreaks.

Diarrheal and other foodborne related infections are prevalent in children in African regions and about 800,000 children die each year from diarrhea and dehydration representing about half of the world estimate (WHO, 1996). The number of scientific evidence-based reported in Table 5 does not, however correspond to the global estimate for foodborne outbreaks in Africa. African governments should endeavor to support the establishment of Laboratories and trained personnel to help in the surveillance of foodborne illnesses so that outbreaks can be documented scientifically for better intervention measures to be taken to reduce the scourge. To achieve an effective and efficient food safety program or culture to tackle foodborne illnesses, African governments must collaborate with both the industries and consumers (Figure 2).

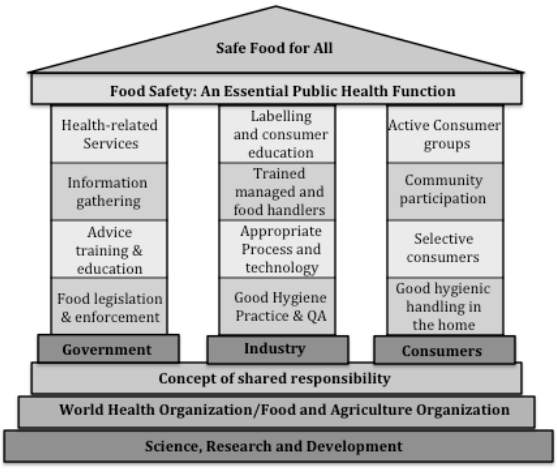

Figure 2. Government, industry and consumers are responsible for food safety.

Source: Käferstein et al., 2003

1.1.3.2 Street foods in Africa

The habit of consuming food or street food outside the home is not uncommon in most African countries (Chauliac *et al*., 1994). Street foods in Africa are normally sold in stationary stalls, opened tables or by hawkers who roam about with the foods to find customers wherever they deem strategic to sell their foods. They are also sold in schools, bus stations, government work places, markets, social events, at places where major construction works is go on, by the windows of vehicles and door to door. In a study conducted in Kenya to determine the role of street foods in the dietary pattern of two low-income groups in Nairobi, it was reported that Street foods play an important role in the diet of poor households in Nairobi (Van 't Riet *et al*., 2001). The situation in Nairobi, like other African cities, is not too different since most of the African population find themselves in the same political and economic milieu.

Inadequate education on food safety issues and the high illiteracy rates of food vendors in African countries have been identified to contribute to poor microbiological quality of food especially street food (Garin *et al*., 2002). In a Routine medical examination of food vendors in secondary schools in Nigeria, it was reported that present practice of medical examination among the food vendors is not enough to ensure food safety (Musa *et al.,* 2002). Regular medical inspections of street food vendors are necessary to prevent the spread of foodborne illnesses from vendors to consumers. However, most street food vendors in Africa do not have the income to perform regular inspection and hence continue to sell food to the public even though their conditions may pose public health threat. Other reported ill health and foodborne related cases associated with the consumption of street foods in Africa were documented in Botswana

(Murindamombe *et al*., 2005), Senegal (Cardinale *et al.,* 2005), South Africa (Lues *et al.,* 2006), and Ghana (Mensah *et al.,* 2002).

1.1.4 Food safety, street foods and food safety culture in Ghana

The government agency responsible for food safety in Ghana is the Ghana Food and Drugs Board (GFDB). The board is the national regulatory body under the Ministry of Health with the responsibility of implementing Food and Drugs Law (http://www.fdbghana.gov.gh/). This board has done its best to perform its mandate since it was inaugurated, but is still faced with logistical problems to carry out proper empirical or scientific-evidence based investigations to support some of its mandates. The administrative problem faced by the GFDB is the duplicated roles that exist between it and the Ghana Standard Boards (GSB), which is responsible for setting standards in the country. Although their roles have been clearly stipulated by the constitution, some loopholes exist in their functions that generate a conflict of interest sometimes.

The sales of street foods in Ghana have been reported to contribute to the reduction of unemployment in the country of which the majority are women and has an annual turnover of over US$ 100 million (FAO/WHO, 2005b). There are some but few publications, about food safety and street foods in Ghana (Mensah *et al*., 2002; Donkor *et al*., 2009; Addo *et al.,* 2011). Generally speaking, the level of food safety culture among Ghanaians is very low in terms of the prevention of foodborne illnesses. In a study carried out by Scoth *et al*. (2007) to determine the hygiene motivator among Ghanaians, the strongest motivations for hand washing with soap among women relate to nurturance, health and for that matter prevention of microorganism on the hand was a weak motivator for hand washing with soap. This report indicates that the microbiological education

among most Ghanaians is very low. Another typical example among Ghanaians is that, although some like their food heated or warmed prior to eating, some do so to improve the palatability of the food, but not to prevent microbes from multiplying in the food. Another example of inadequate knowledge on microbes in Ghana is that, some Ghanaians prefer to wash their hands with soap after eating food rather than before. The reason for this behavior is aesthetic rather than having the hands free of microbes before eating since they do not want their hands to be foul smelling after eating. A microbiological meta-analysis of street foods publications in Ghana is explicitly analyzed in the first publication that constitutes this thesis. Figure 3 illustrates the general situation of street foods in Ghana.

Figure 3. General pictures of street foods in Ghana. (A) Hawking street food vendors selling various types of food by a vehicle (B) A stationary street food vendor selling on a table in a market (C) Hawking street vendors selling fried snail by the roadside (D) A typical stall from which street foods are sold (E) A street food being served with the bare hand (F) A typically saved street food called "waakye" with macaroni and tilapia fish (G) A group of women preparing a favorite food in Ghana called "fufu" (H) A group of women saving food in a typical restaurant popularly called 'Chop Bar' in Ghana (I) A customer washing his hand to eat "fufu" with his bare hand.

1.2 Food Safety Versus Food Security and in Africa

Unlike the definition of Food Safety in the first paragraph of this thesis, Food Security is the availability, accessibility and utilization of food to human. We must ensure all the three keywords to have food security because availability, for example, does not mean that the food is accessible or appropriate for utilization. The relationship between food safety and security can also be elucidated by this definition by Labadarios *et al.* (2011) that food security as an umbrella term

includes, (i) the availability of food that is nutritious and safe; (ii) an assured ability to procure and acquire food of good quality in a socially acceptable way (e.g. without resorting to emergency food supplies, scavenging, stealing or similar coping strategies). Food safety and security are therefore, linked directly or indirectly. Both are geared towards the provision of healthy life hence they are strongly linked. For example a person who is food in-secured is more likely to consume unwholesome food than one who is food secured. The food insecurity situation in Africa therefore affects its food safety situations.

Some decades ago, it was observed that food safety was a reserved privilege of the industrialized countries, who have enough and hence can think of choosing the best to prevent foodborne illnesses rather than the developing countries where majority did not know where the next meal was coming from (Käferstein, 2003). African governments must take into account that once they remain heavily importing countries, it is imperative to develop food safety programs that are capable of detecting undesirable elements that may be detrimental to the health of it populace. Good food safety programs will enhance the credibility and acceptability of African countries on the international market and hence their surplus produce can be marketed to generate income for poor farmers allowing them to enjoy the fruit of their labor. This means that despite the problem of food insecurity in Africa, the governments, non-governmental organizations and individuals must ensure that food safety is as high on their agenda, in order to prevent foodborne illness. The fear is that food safety may be another major problem in the future even if food insecurity is achieved. The two interventions must therefore go hand in hand.

1. 3 Malaria Illnesses Versus Food safety and Foodborne Illnesses in Africa and Worldwide

Reports from WHO indicates that there were an estimated 655,000 malarial deaths in 2010 of which 91% (596,050) were in Africa; approximately, 86% of malarial deaths globally were children under 5 years of age and international funding for malaria was expected to peak in at US$ 2 billion in 2011 (WHO, 2011). The Global Fund to Fight AIDS, Tuberculosis, and Malaria was established in 2002 to fund substantial and effective interventions to reduce infection, illness, and deaths globally. As of December 2006 the Global Fund has committed $2.6 billion over 5 years to support malaria prevention and control in 85 countries (Nahlen *et al.*, 2007).

However, using the WHO's estimated figure for 2008 due to the unavailability of estimated data (new data yet to be released in 2012) since then, it has been estimated that 2.2 million deaths are attributable to diarrheal disease (a considerable proportion are foodborne), of which 1.8 million occur in children less than 5 yrs. of age; overall, it is estimated that 1.5 billion cases of diarrheal disease occur annually in children under 5 yrs. of age (WHO 2008b), of which the majority of the deaths occur in developing countries. Moreover, foodborne diseases appear to be increasing globally and health officials seem overwhelmed by the situation (WHO, 2007c). Unlike the Global Fund for Malaria eradication, the world's first Global Food Safety Fund was not launched until 2011(http://fscf-ptin.apec.org). Figure 4 illustrates the disparity between: global and African foodborne illnesses/global funds committed to food safety, and global and Africa malarial deaths/ global funds committed to malaria.

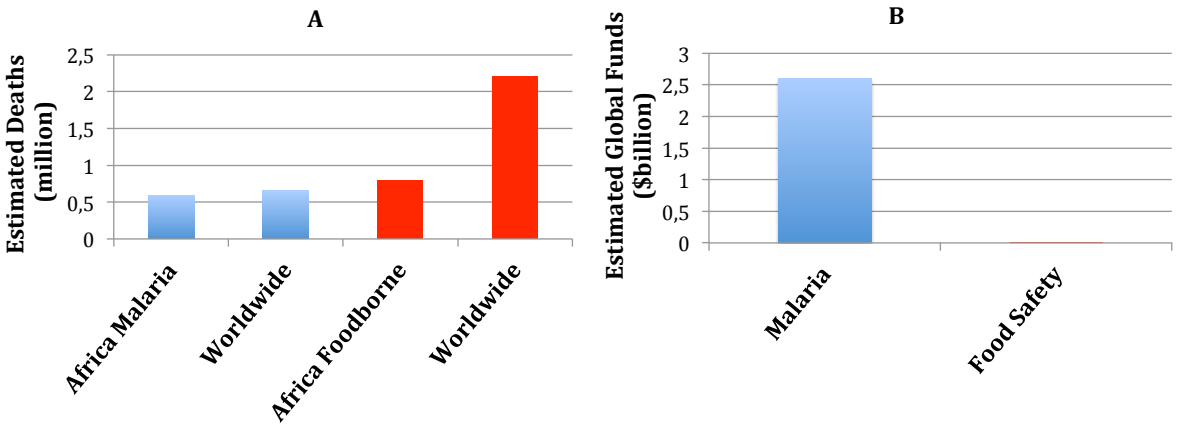

Figure 4. Estimated disparities between Foodborne illnesses and Malaria based on current available data (A) Under 5 mortality in Africa as a result of malaria versus total malaria deaths worldwide (2011) and under 5 mortality in Africa as a result of foodborne illnesses versus the foodborne illnesses related deaths worldwide (2008). (B) Estimated global funds for malaria eradication versus global funds for food safety (2011).

1.4 Consumer Awareness in Food Safety Issues in Africa

Generally, the activities of consumer awareness associations in food safety are very low and even in countries where they exist, they are very ineffective and inefficient because some consumer associations do not have the expertise to scientifically prove their concerns. There are various consumer protection associations in individual countries (South African National Consumer Union, Consumers Association of Ghana, Consumer Protection Council, Tanzania Fair Competition Commission, Zambia Consumers Association etc.) but there is not yet any strong consumer protection association in Africa that connects all the countries. The formation of such unified groups or associations among African countries are necessary because an unwholesome food that enters one country can get it way to the other countries. There was however, an initiative in 2009 called African Consumer Protection Dialogue among African governments, NGOs and the US Federal Trade commission that seeks to link African consumers to the rest of the world (https://icpen.org/).

1.5 The application of Hazard Analysis Critical Control Point, and Microbiological Risk Assessment Based Research in Food safety in Africa

1.5.1 Hazard Analysis and Critical Control Point

This concept was developed in the 1960s in the United States of America in order to produce safe foods for the space program. The Hazard Analysis Critical Control Point (HACCP) is a proven approach, which has been adopted by various stakeholders in the food safety sector to ensure the safety of food till it reaches the final consumer (Majewski, 1992; Baker *et al.,* 1995). This approach, although very important, is not commonly employed in most African countries probably because of inadequate experts as well as equipment and materials. The approach is a very easy and a cost effective measure to reducing microbial hazards both at the local level (eg. Street foods) and large scale factories at the national level. Even though at a slow rate, the HACCP has been employed in certain areas in Ghana (Amoa-Awua *et al.,* 2007) and other African countries (Ababouch, 2000; El-Tawila *et al.,* 1997; Jagals *et al.,* 2004; Kassem *et al.,* 2002).

1.5.2 Microbiological risk analysis based research

The concept of microbial risk analysis has three components (a) risk assessment (b) risk management and (c) risk communication. The risk assessment component is divided into qualitative risk analysis and quantitative risk analysis (QRA). Quantitative risk assessment techniques were originally developed for evaluating the risk associated with exposure to chemical hazard in 1983 (NAS, 1983). The process of microbial risk assessment consists of (i) hazard identification (ii) exposure assessment (iii) dose-response assessment and (iv) risk characterization. There is increasing interest in the application of QRA in the production of microbiologically safe food products. This model is a probabilistic

one that seeks to determine the risk of contamination of food at various stages of food production (Cassin *et al.,* 1998). The QRA is also employed by both national and international bodies to ensure that there is minimal contamination in the food chain (Hoorstra *et al.,* 2001). Most of the measures that are now taken by both international and national governments to prevent or curb foodborne diseases rely on both QRA and HACCP. However, risk assessment based analysis in ensuring safe food and the prevention of foodborne illness is limited in Africa. Only very few works involving quantitative microbial risk assessment are available in Africa (Steyn *et al.,* 2004; Seidu *et al.,* 2008; Labite *et al.,* 2010).

1.6 Meta-analysis

Meta-analysis can be used to assess the generalized outcome of specific interventions or studies in order to arrive at common but important solutions that will improve the success of the interventions of studies (Brockwell *et al.,* 2001). This must be done with objectivity and must precisely evaluate the shortcoming of each individual study (Egger *et al.,* 1997). Although there are several interventions both at the national and international to reduce foodborne diseases, very little work exists on meta-analysis on food safety and foodborne illness research in Ghana as well as the whole of Africa. In the first publication that constitutes this Thesis, meta-analysis was used to analyze the microbiological food safety publications to see their overall effect on food safety and foodborne illnesses in Ghana.

2.0 *Salmonella*

2.1 A brief history of *Salmonella*

Infections caused by *Salmonella* might have existed since the ages, but man's ability to recognize or discover the destructive activity of this bacterium was only in the past thirteen decades. Although it was not directly recognized for causing typhoid fever, it was reported that the death rate as a result of typhoid fever declined to about 50% due to improved sanitation in Great Britain after successfully passing the Public Health Act in 1875 (Swith, 1955 cited by Yoshikawa, 1980). This shows that sanitation has played a very crucial role in the reduction of infections caused by *Salmonella* in the past centuries. The present name of the genus *Salmonella* was named after Daniel Elmer Salmon (1850-1914), an American veterinary pathologist, who was the leading investigator for the causative agent of hog cholera, a disease affecting pigs. However, it was his colleague and subordinate Theobald Smith who first discovered the bacterium in 1885, in pigs (Salmo *et al.,* 1885). The first organism isolated by the group was *Salmonella enterica* (formerly called *S. choleraesuis*). Not long after the discovery of *Salmonella enterica*, thus, as far back as the early 1900s, *Salmonellae* were implicated in a number of foodborne outbreaks even though little knowledge exists of some of the outbreaks (Savage *et al.,* 1908a, 1929b). Most cases were not strongly supported because of the limited scientific knowledge that existed compared to the present day technologies. *Salmonella* serotypes were found in the following decades to be involved in numerous animal and human diseases (Steele, 1963). *Salmonella* is an important pathogen both for humans and animals and causes severe infections.

2.2 Biochemistry, Taxonomy and Serovars

Salmonella organisms are members of the family Enterobacteriaceae. They are non-spore forming aerobic, facultative anaerobic, Gram-negative bacilli, non-lactose fermenting and possess motile peritrichous flagella. The cell wall is a complex structure composed of lipids, polysaccharides, proteins and lipoprotein (Lüderitz, 1966). Most species produce hydrogen sulfide, which can readily be detected by growing them on media containing ferrous sulfate (Clark *et al.*, 1987) such as Kliger iron agar, triple sugar iron agar etc. *Salmonella* consists of two species namely *Salmonella enterica* and *Salmonella bongori* of which the most frequently pathogenic serovar that affect warm-blooded animals belong to the serovar *S. enterica* while *S. bongori* is mostly associated with reptiles but cause disease in humans (Fookes *et al*., 2011). *Salmonella enterica* is divided into 6 subspecies namely *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica* (Tindall *et al.*, 2005). Currently, *S. enterica* subspecies are further subdivided into over 2,500 serovars (Clayton *et al*., 2008).

The differences within *Salmonella* serovars are based on the differences in surface O and H antigens. The O antigens are somatic and derived from the polysaccharide domain of lipopolysaccharide (LPS) in the cell wall while the H antigens are derived from the flagellin protein in the flagella. The antigens are identified by slide or tube agglutination test using specific anti-sera. The Vi capsular polysaccharide antigen is also characteristic in the identification and virulence of *Salmonella enterica serovar* Typhi (Sharma *et al.,* 2004). There are also serovars specific real and conventional PCR's for the identification of *Salmonellae* (Arya, 1994; Sha *et al.,* 1997; Lungu *et al.,* 2012).

2.3. Salmonellosis in Africa

Salmonellosis is an infection that is caused by the bacteria from the genus *Salmonella*. Although Salmonellosis is a threat to public health worldwide, estimates of the disease burden from African countries remains deficient because of the little data that comes from African countries (Mweu *et al.,* 2008). For instance the global burden of disease estimates for typhoid were based on a total of 22 community-based incidence studies with 19 from continents other than Africa and only 3 out of 54 countries from Africa (Crump *et al.,* 2004). Although *Salmonellae* infections might have existed in Africa before its discovery elsewhere, the preliminary scientific detections of infections caused by *Salmonella* in Africa probably started in South Africa (Reaud, 1947), and later in other countries in the continent (Nicolle *et al.,* 1955; Crocker, 1957; Hughes, 1958). Higher infection rates of *Salmonella* have been estimated in African countries annually, but most of the cases are not documented with scientific evidence because of inadequate effective diagnostic systems, logistics and personnel (Kiriuki, 2008). The estimated cases especially for Africa and other resource-limited countries are woefully under-estimated. Human Salmonellosis has been observed to represent 1 to 10% of the real incidence of the disease (Oosterom, 1991). As of 2007 only 6 countries in Africa were part of the Global *Salmonella* Surveillance Network of the (WHO, 2007b). The relatively low number of African countries involved in the reporting of salmonellosis to WHO also may impede the estimation of cases at the global level. In the Northern African countries, Egypt, Morocco, Libya, Tunisia, Algeria, typhoid fever is said to be endemic with high mortality rates among infants with congenital anomalies (Ghenghesh *et al.,* 2009). The problem of typhoid in Eastern Africa with reference to a recent review

carried out in Ethiopia reported a high prevalence of salmonellosis in the region (Beyene *et al.,* 2008). In a recent study carried out in a pediatric hospital in the Democratic Republic of Congo, among the 1,528 children included in the study, 26.8% were bacteremic and *Salmonella* accounted for 59% of all bloodstream infections (Vandenberg *et al.,* 2010). In Western Africa, a recent cross-sectional study carried out in Benin showed that there was high prevalence of salmonellosis in that country with *S*. Typhi (94.8%) isolated in most of the cases (Dovonou *et al.,* 2011) and similar reports were obtained from Ghana (Gross *et al.,* 2011). In South Africa, an increasing number of infections by invasive non-typhoid *Salmonella* (iNTS) were confirmed during the period of 2003 and 2004 while similar confirmations were also reported in Malawi within the period 1998-2004 (Feasey *et al.,* 2010). A recent study by a team of CDC-Kenya have documented rates of typhoid fever in urban Nairobi, Kenya and reported higher incidence of typhoid among children age 2-4 years as compared to their Asian counterparts (Chris *et al.,* 2011).

There are increasing reports of non-typhoid *Salmonella* (NTS) in some African countries (Ben *et al.,* 1993; Beyene *et al.,* 2008; Mtove *et al.,* 2010). In a recent work done on NTS in Ghana it was observed that in sub-Saharan Africa, NTS were the predominant organisms isolated from children with clinical pneumonia (Schwarz *et al.,* 2010). In a recent publication in Kenya, NTS was reported to be the leading cause of bacteremia in rural Kenya (Tabu *et al.,* 2012). Due to the weak immune system of HIV patients and the high prevealence of HIV in the continent, cases of NTS have also been investigated in HIV patients in some African countries. In a study conducted in Malawi, it was concluded that iNTS have established a new and emerging pathogenesis in the context of HIV infection

in Africa (Gordon *et al.,* 2010). It was also reported in a study that NTS would be one of the major threats to HIV positive patients (Aubry *et al.,* 1992). Surprisingly, there have not been any reports of a reduction of diseases caused by *Salmonella* in any country of the continent. If there were appropriate means of detecting *Salmonella* in Africa, one can be very sure that higher and intriguing figures would have been obtained much higher than in the aforementioned studies.

The high reported cases may be due to inadequate knowledge on microorganisms. This leads to poor hygienic practices, which may lead to a large number of the population becoming either carriers or infected. In a study conducted in Tamale, the capital of the Northern Region of Ghana to asses the perception of the people towards anthrax, Opare *et al.* (2000) reported that many of the respondents attributed the disease to supernatural sources. Meaning that people have still not come to the realization that microorganisms cause diseases. Figure 5 shows some pictures of predisposing factors of the African population to Salmonellosis and other infectious diseases.

Figure 5. Predisposing factors to high rate of Salmonellosis and other infectious diseases in Africa (A) A farmer drinking water from a stream, which is used by both cold and warm blooded animals. Some of these streams pass through national parks with different types of wildlife. These animals bath, drink, defecate and urinate in the streams before being used by humans without treatment (B) A teenager roaming with beef in a market in desperation for customers. The animals are normally slaughtered in the house without any pre or post slaughter inspection. The beef is sold uncovered for several hours under the scorching sun and unpaved market (C) Children in an open kitchen with uncovered bowls with roaming sheep and donkey. These animals litter the kitchen with their feces and drink from the opened bowls, which may be used later on for domestic purposes (D) Feces in an open defecation zone where people defecate in a forest near a town center. These feces are normally carried in surface running water, which pass through houses and finally end up in gutters or dugouts for animal and human use as for the irrigation of fresh leafy vegetables.

2.4 Brief Global Epidemiology and Virulence Factors and Pathogenicity

2.4.1 Brief global epidemiology

The incidence of Salmonellosis especially the NTS has been reported to be the most predominant form of *Salmonella* infections globally and many countries have included them in their surveillance (Chiu *et al.,* 2004). The bacteria are transmitted to humans through the consumption of contaminated food or water. The first symptom may appear 12-17 hours after infection. The initial symptoms

may include fever, abdominal pain or cramps, diarrhea, nausea and may last for 4–7 days. Excretion of *Salmonella* after infection could continue for weeks (Buchwald *et al.,* 1984). Even though Salmonellosis is not well documented in Africa and other developing countries in Latin America, the Caribbean and Asia, the estimates for the developed world may be more close to the real situation. In the US, for instance, it is estimated that NTS is the second leading cause of illness and the leading cause of hospitalization and death of foodborne illness (Scallan *et al.,* 2011). Although salmonellosis was one of the leading causes of outbreaks in the European Union, EFSA reported a decrease in the prevalence of the diseases (EFSA, 2012). The prevalence of salmonellosis has also been reduced in New Zealand (MAFA, 2011). A study carried out to estimate the most common serovars globally reported that, *Salmonella* serovars Enteritidis and Typhimurium ranked as the most common and second most common serovar, respectively (Hendriksen *et al.,* 2011). Table 6 shows data of *Salmonella* surveillance from 3 African countries and the topmost 20 frequently isolated serovars. The latest scientifically documented outbreak of Salmonellosis in Africa (caused by *S*. Typhi) was reported between October 2004 and January 2005 in Democratic Republic of Congo, where the case fatality rate was 53% (Muyembe-Tamfum *et al.*, 2009).

Table 6. Distribution of the most frequently Serotyped human *Salmonella* Isolates from African Countries in numbers and percentages

Country	Cameroon[a]				Senegal				Tunisia			
Population	**18,467,692**				**12,853,259**				**10,383,577**			
Year	**2001**	**2003**	**2005**	**2007**	**2001**	**2003**	**2005**	**2007**	**2001**	**2003**	**2005**	**2007**
Total serotyped	**242**	**182**	**151**	**99**	**232**	**176**	**151**	**102**	**720**	**599**	**363**	**243**
Serotypes among top 20 in region (%)	**98.8**	**87.2**	**69.5**	**55.6**	**42.2**	**57.4**	**44.2**	**29.4**	**86.8**	**79.5**	**94.4**	**89.3**
Enteritidis	20.7	14.8	21.2	19.2	19.8	8.0	19.2	4.9	14.3	27.9	28.1	49.0
Typhimurium	71.9	53.3	28.5	18.2	4.3	5.1		10.8		4.0	7.4	11.5
Livingtone									21.1	26.7	5.5	
Corvallis						1.7	2.6		14.0	3.5	6.1	
Typhi	5.4	17.0	18.5	16.2	7.8	8.5	7.9	4.9				2.1
Braenderup				1.0					13.1		3.6	
Anatum									4.3	3.2	11.0	4.9
Infantis		0.5							5.7		10.2	1.6
Kentucky					6.0	9.7	4.0	3.9		2.8		6.2
Cerro									4.3	3.2	1.1	
Newport									2.5	2.7	5.2	
Mbandaka	0.8							2.0	5.3		2.8	
Amsterdam										3.0	3.3	6.6
Hadar		1.6	1.3	1.0	4.3	1.7	2.6	2.9		2.5		
Tokoin						17.6						
Zanzibar											4.1	4.1
Altona											4.1	2.1
Muenster						1.1	2.6				1.9	1.2
Wien									2.2			
Bredeney						4.0	5.3					

[a]*Institutional data*
Source: Hendriken et al., 2011

2.4.1.1. Epidiomology in Ghana

Although Salmonellosis may be very frequent in Ghana, there exist only a few scientifically reported cases (Hughes, 1958; Newman, 1996; Feglo *et al.,* 2004; Schwarz *et al.*, 2010). As showed in figure 4 and 5 above, there is no doubt that the prevalence of Salmonellosis is high in Ghana. In Ghana, and in the Northern Region of Ghana especially, the access to portable water is very limited to the population (DHS, 2003) and most of the people in the rural areas of the Northern Region resort to dugout wells for water for domestic consumption (Cobbina *et al.*, 2010). Some of the dugout wells also serve as sources of drinking water for

domestic and wild animals and reptiles, which are known reservoirs of *Salmonella. Salmonella* surveillance in the area is non-existent and there is no report that links the prevalence of Salmonellosis to the dugout wells, which are being shared by humans and animals. Outbreaks of Salmonellosis are rarely reported and the existing information on the prevalence of *Salmonella* in the region was not outbreak related (Djie-Maletz *et al.,* 2008). The study conducted by Djie-Maletz *et al.* (2008) among children had a very low *Salmonella* carriage rate among both healthy and symptomatic ones. There are no reports about the prevalent serovars in the Northern Region of Ghana and little data exist on the prevalence serovars in Ghana. In the second publication that constitutes this Thesis, a research was done in Northern Ghana to determine the prevalence of *Salmonellae* and their resistance to commonly used antibiotics in the region.

2.4.2 Pathogenicity and Virulence factors

Salmonella is an invasive, facultative intracellular pathogen that is adapted to many of its host's immune cells and has many mechanisms to by-pass the defence mechanisms of the immune system and causing severe damage to host cells (Jantsch *et al.,* 2011). Pathogenicity-associated Islands consist of large regions or mobile genetic elements of genomic DNA that are present in pathogenic bacterial strains but absent from the genomes of non-pathogenic members of the same species (Schmidt *et al.,* 2004). The major *Salmonella* Pathogenicity Islands (SPIs) include SPI-1, SPI-2, SPI-3, SPI-4 and SPI-5 (Rychlik *et al.,* 2009). However, at present, 19 different SPIs have been described that encode the most prominent virulence phenotypes that are involved in host-cell invasion and intracellular pathogenicity (Vernikos *et al.,* 2006: Fuentes *et al.,* 2008: Blondel *et al.,* 2010). They encode type III secretion systems (T3SS) that

form syringe-like organelles on the surface of gram-negative bacteria and enable the injection of effector proteins directly into the cytosol and aid in the formation of *Salmonella* containing vesicle in eukaryotic cells (Agbor *et al.,* 2011). SPI1 promotes the invasion of non-phagocytic intestinal epithelial cells and the initiation of the inflammatory responses in the intestines (Hapfelmeier *et al*, 2004). The T3SS encoded by SPI1 and 2 are major virulence factors of *Salmonella* (Dieye *et al.,* 2009). Figure 6 shows a picture of *Salmonellae* invading human cells.

Figure 6. *Salmonella* Typhimurium (red) invades cultured human cells in this color-enhanced scanning electron micrograph. *Source: Rocky Mountain Laboratories, NIAID, NI*

3.0 *Escherichia coli*

3. 1 A Brief History of *Escherichia coli*

The organism *Escherichia coli* (*E. coli*) (formerly called *Bacterium coli commune*), was discovered by the German pediatrician Theodor Escherich in 1885 from the faces of infants (Escherich, 1885). *E. coli* were initially considered to be harmless since it was first isolated from healthy infants. However, it was known later in 1920s that the organisms include some severe pathogenic strains that caused gastroenteritis (Adam, 1923, cited by Braun, 1974), even though a large majority is harmless. These organisms are part of the normal flora of warm-blooded animals. In the first few hours of life *E. coli* is not part of the normal flora but it later colonizes the gastrointestinal tract. *Escherichia coli* is by far the most studied microorganism in the history of microbiology and biology. The K-12 strain of *E. coli*, was used as the prototype for genetic and molecular works because of its suitability for genetic analysis by recombination (Lederberg, 1947). Although a lot has been studied about this organism over the last decades, there is a lot more to learn, especially about its genome plasticity and its ability to mutate and become very pathogenic in both humans and animals (Bezuidt *et al.,* 2011; Chauduri *et al.*, 2012; Künne *et al.,* 2012). However, only a very small portion of studies has been done on *E. coli* in African countries. A search on PubMed with the search word '*E. coli*' and '*E. coli* Africa' showed that only 0.6% of the articles had something to do with *E. coli* in Africa.

Kingdom	***Bacteria***
Phylum	***Proteobacteria***
Class	***Gammapreoteobacteria***
Order	***Enterobacteriales***
Family	***Enterobacteriaceae***
Genus	***Escherichia***
Species	***E. coli***

3.2 Taxonomy, Biochemistry and Serotypes

The taxonomy of *E. coli* as shown below has also undergone a series of changes before the present name was accepted. Its name after its discovery was *Bacterium coli commune* but was later changed in honor of its discoverer Theodor Escherich (Castellani *et. al*., 1919). They are a gram negative, rod-shaped, non-spore forming, facultative anaerobic organism and most possess peritrichous flagella. The organisms ferment the following sugars: glucose, arabinose, mannitol, sorbitol, xylose and are motile at 36°C. They ferment lactose in MacConkey agar to produce lactic acid. They are catalase positive and oxidative negative. Under the indole, methyl red, Voges-Prokauer, and citrate test (IMViC test), the organism is positive, positive, negative and negative respectively. Other species of the genus *Escherichia* are *E. hermanii, E. fergusonii, E. vulveris, E. blatttae* (Farmer, 1999) *and E. albertii* (Abbott *et al.,* 2003). *E. coli* is positive in beta glucuronidase identification assay due to the presence of the beta glucuronidase enzyme activity in *E. coli* (Killan *et al.,* 1979; Rice *et al.,* 1990), but the serotype *E. coli* O157:H7 does not ferment sorbitol and is negative to beta glucuronidase (Eppinger *et al.,* 2011)

There are different serotypes of *E. coli* based on the O (lipopolysaccharides), H (flagella), and K (capsular) antigens by agglutination with polyvalent or monovalent antisera. There are several serotypes about 50,000-100,000 due to the high diversity of the antigens present among the genus (Orskov *et al.,* 1992). There are also molecular methods for serotyping that are based on the amplification of specific genes of the O, H and K antigens by PCRs (Machado *et al.,* 2000; DebRoy *et al*., 2004; Fratamico *et al.,* 2011) or based on PCR of the beta glucuronidase genes (Heininger *et al.,* 1999; Pavlovic *et al.,* 2010). In general,

there are some serotypes (i) that are not implicated in infections (commensals) (ii) implicated in the cause of acute diarrhea and related diseases in the gastrointestinal tract, called intestinal pathogenic *E. coli* (IPEC) and (iii) implicated in the cause of diseases outside the gastrointestinal tract after acquiring some virulence factors that enable it to become invasive and permeate the intestines and attack other organs in the body, called extra-intestinal pathogenic *E. coli* (ExPEC). There also exist the Avian pathogenic *E. coli* (APEC) serorotypes. The difference between the normal and pathogenic *E. coli* serotypes is the acquisition of virulence factors by the pathogenic serotype (Menrath *et al.,* 2010). *Escherichai coli* O157:H7 producing Shiga toxin and several non-O157:H7 Shiga toxin producing serotypes have been implicated in most Hemolytic uremic syndrome cases (Scheiring *et al.,* 2008). The non-O157:H7 serotypes mostly associated with Shiga toxin production are O26, O45, O103, O111, O121 and O145 (Fratamico *et al.,* 2011). The serotype O104:H4 has also been involved in some infections (Bae *et al.,* 2006; Buchholz *et al.,* 2011) as well as the pandemic *E. coli* sequence type 131 (*E. coli* ST131) (Poma *et al.,* 2009; Lavigne *et al.,* 20102). The recent new strain of pathogenic *E. coli* from Germany in 2011, 0104:H4 possessed the characteristics of two serotypes (pathotypes) (EAEC and EHEC) and at the moment called Enteroaggregative Hemorrhagic *E. coli* (EAHEC) (Brzuszkiewicz *et al*., 2011). The nomenclature of pathogenic *E. coli* is complex and depends on factors such as the pathogenicity, virulence or invasiveness of the strain that is involved or the type of organs they attack. Table 7 shows the nomenclature of the different toxin sub-types of diarrheagenic and enterohemorrhagic *E. coli.*

Tabal 7. Nomenclature of Diarrheagenic *E. coli* and Enterohemorrhagic *E. coli* sub-types

Nomenclature		**Description**
Abbreviation	**Meaning**	
DEC	Diarrheagenic *Escherichia coli*	Any group of *E. coli* which has been associated with the ability to cause diarrhea
DEAC	Diffusely-adherent *Escherichia coli*	A group of *E. coli* which has been associated with the ability to cause diarrhea defined by a specific pattern of adherence using the HEp-2 cell assay
EAEC	Enteroaggregative *Escherichia coli*	A group of *E. coli* which has been associated with the ability to cause diarrhea defined by a specific pattern of aggregation using the HEp-2 cell assay
EIEC	Enteroinvasive *Escherichia coli*	A group of *E. coli* which has been associated with the ability to cause diarrhea defined by the presence of invasion genes also present in *Shigella*
EPEC	Enteropathogenic *Escherichia coli*	A group of *E. coli* which has been associated with the ability to cause diarrhea originally defined as specific serotypes and by a specific pattern of adherence using the HEp-2 cell assay but now by the presence of certain virulence factors including the locus enterocytes effacement and associated effectors
ETEC	Enterotoxigenic *Escherichia coli*	A group of *E. coli* which has been associated with the ability to cause diarrhea defined by the presence of heat stable or heat labile toxin
VTEC	Verocytotoxic *Escherichia coli*	A group of *E. coli* which has been associated with the ability to cause diarrhea defined by the presence of a toxic gene, *vtx,* which has activity against cultured vero cells.
STEC	Shiga Tosin-Producing *Escherichia coli*	A group of *E. coli* which has been associated with the ability to cause diarrhea defined by the presence of toxin gene, *stx,* because of genetic similarity with toxin of *Shigella dysenteriae*
stx*/*vtx	Toxic genes	For E. coli these two genes are synonymous - only in *Shigella dysenteriae* type 1 is *stx* used exclusively. The discussion about which name revolves around the scientifically agreed use of the same gene name for genes which show homology (shared ancestry); *vtx1* and *stx* but for *vtx2*/*stx* this may not be true
EHEC	Enterohemorrhagic *Escherichia coli*	VTEC/STEC patients that have the symptom of bloody diarrhea/ hemorrhagic colitis. This infection can lead to hemolytic uremic syndrome (HUS) characterized by acute renal failure, hemorrhagic anemia (anemia due to hemolysis) and thrombocytopenia (low number of platelet).
***stx*1/*vtx*1**	Toxin gene type 1	Several genetic variants including *vtx*1a, *vtx*1c, *vtx*1d
***Stx*2/*vtx*2**	Toxin gene type 2	Several genetic variants including *vtx*2a, *vtx*2b, *vtx*2c, *vtx*2d, *vtx*2e, *vtx*2f and *vtx*2g

The terms stx/vtx or STEC/VTEC are used interchangeably
Source: Chattaway et al., 2011

3.3 Use as Therapeutic, Biotechnological and Model organism

Although some few strains from this genus are involved in chronic diseases or severe foodborne illness outbreaks, some beneficial strains have been put into different uses for the benefit of both plants and animals. *E. coli* has been demonstrated to synthesize Vitamin K and B12 in the gut of humans to the benefit of humans and animals (Benthley *et al.,* 1982). The *E. coli* strain Nissle 1917 (EcN or mutaflor) is used as a probiotic for the treatment of chronic inflammatory diseases (Schultz, 2008; Güttsches *et al.*, 2012) because of its inhibition activity of *Salmonella* (Mandel *et al*., 1995; Schierack *et al.,* 2011) and *Candida albicans* (Hockertz *et al.,* I997) in vivo as well as in vitro suppression of *Salmonella, Yersinia enterocolitica, Shigella flexneri, Listeria pneumophilia, Listeria monocytogenes* (Altenhoefer *et al.,* 2004). The EcN has also been investigated to produce significant inhibitory effects on adherent-invasive *E. coli* and can also be used in preventive or curative therapies in patients with Crohn's disease (Boudeau *et al.,* 2003).

In the field of biotechnology, *E. coli* has been used in recombinant technologies to engineer the biosynthesis of proteins such as insulin for the treatment of diabetic patients (Chan *et al.,* 1981; Raptis *et al.,* 1985; The, 1989), growth hormones for humans, animals and plants (Kato *et al*., 1987; Cheng *et al.,* 1995; Mukhopadhyay *et al.,* 2002), human factor VIII antibodies for hemophiliacs (Scandella *et al*., 1988) and many antibodies (Humphrey, 2003; Willoughby *et al,* 2004). *E. coli* has recently been engineered to biosynthesize glycans (Valderrama-Rincon *et al.,* 2012). The organism has also been modified to synthesize products such as subunit vaccines, epitopes, metal-binding motifs and immobilized enzymes (Cornelis, 2000). *E coli* has also been modified metabolically for

homolactate fermentation and other fermentation processes (Zhu *et al.,* 2007; Lau *et al.,* 2010).

In the field of microbiology, the laboratory strain *E. coli* K-12 especially has been widely used as a model organisms for many experiments. They are used in biofilm experiments (Kétyi, 1989; White-Ziegler, *et al.,* 2008). They are widely used as models in conjugation experiments (San-Millan *et al.,* 2009; Escudero *et al*., 2011) as well as evolutionary experiments (Mori *et al*., 2009; Korolev *et al.,* 2010).

3.3.1 Use as indicator

E. coli was introduced in the I980s as an indicator to monitor drinking water supplies as well as a marker for the microbiological safety of food (Mossel *et al.,* 1995). This organism has been used extensively in the determination of the microbiological quality of water and food till today (Viau *et al.*, 2011). Their presence in water and food is considered a fecal contamination. The use of *E. coli* as an indicator organism is advantageous to especially those in resource-limited countries because of the ease of its isolation and identification using the conventional techniques. For the surveillance of antibiotics in food, *E. coli* is employed as an indicator organism in the determination of antibiotic residues in animal tissues (Choi *et al.,* 1999; Valtonen *et al.,* 2002).

3.4 A Brief General Epidemiology

3.4.1 Symptoms and complications

Diseases caused by *E. coli* (gastroenteritis) had been documented since the 1920s (Adam, 1923, cited by Braun, 1974). The symptoms of pathogenic *E. coli* infection is characterized by fever, abdominal cramps, diarrhea, some cases may

result in bloody diarrhea; the incubation period ranges from 3-8 days but most people recover within 10 days after infection. Other symptoms as a result of pathogenic *E. coli* may be due to the specific serotypes involved in the etiology of the disease as described above (Table 7). The complications caused by pathogenic *E. coli* range from hemolytic uremic syndrome (HUS) or acute renal failure, hemorrhagic colitis, (Mahon *et al.,* 1997; Siegler *et al.*, 2003; Bezuidt *et al.,* 2011), Urinary tract infections (UTI) resulting in cystitis or pyelonephritis, septicemia or meningitis (Donnenberg *et al.,* 2001). HUS may also result in thrombocytopenia (Blaser, 2004), which is also associated with the kidney. It can cause nosocomial pneumonia, cholangitis, cholecystitis, peritonitis, cellulitis, osteomyelitis or arthritis infections (Pitout, 2012). The most affected people by HUS are young children, elderly people and the immunosuppressed (Gould *et al.,* 2009).

3.4.2 Mode of transmission

The primary or principal source or reservoir is healthy cattle; food of bovine origins including beef, milk, and dairy products (Chapman, 2000; Jacob *et al.,* 2011). Domestic and livestock animals, wildlife, amphibians, fish and invertebrates are known to be carriers of pathogenic *E. coli* stains (Ferens *et al.,* 2011). Transmission of pathogenic *E. coli* strains to people occurs primarily via ingestion of inadequately processed food or water or contact with infected people. A typical example of transmission through food was the association of sprout with the German outbreak in 2011, one of the largest outbreaks caused by pathogenic *E. coli* in history (Buchholz *et al.,* 2011).

3.4.3 Prevalence and incidence worldwide

Currently, there exists no estimate of the global burden of pathogenic *E. coli,* but there is quite a comprehensive surveillance in the USA and Europe due to the availability of resources for investigations. The estimates of incidence or prevalence in Africa, Asia and Latin American countries will surely not be precise due to records of only sporadic outbreaks instead of continuous surveillance programs. A surveillance report from the US by Gould *et al*. (2009) reported that death occurred in 0.6% of all patients with STEC O157 infection and in 4.6% of those with HUS. This is quite high and alarming. In the EU report on the trend of zoonoses and foodborne outbreaks in 2010, higher number of verotoxigenic *E. coli* (VTEC) infections were reported and this number has been increasing since 2008 (EFSA, 2012). In 2009, 35 patients were involved in an outbreak of food poisoning in Japan due to enterohemorrhagic *E. coli* O157:H7 (Watanabe *et al.,* 2010). Reports as a result of pathogenic *E. coli* were also recorded in other Asian countries such as Iran (Bonyadian *et al.,* 2010), China (Li *et al.,* 2011), South Korea (Kim *et al*., 2010), India (Surendraraj *et al.,* 2010). In South America there are reports from Peru (Contreras *et al.,* 2011), Colombia (Rúgeles *et al.,* 2010), Argentina (Rivero *et al.*, 2010), and Brazil (De Toni *et al.,* 2009). The prevalence of pathogenic *E. coli* seems to reach a global level based on the above reported cases from the different continents.

3.4.3.1 Prevalence and incidence in Africa

The real burden of EHEC in African countries is difficult to estimate as a result of inadequate laboratory resources, personnel, material or difference in methodology (Okeke, 2009). The first prevalence investigation into pathogenic *E. coli* in Africa probably started in Ghana (Agbodaze *et al*., 1988), however,

surveillance has not been sustained since then. The largest report of outbreak of pathogenic *E. coli* (*E. coli* O157:H7) was probably recorded in 1992 from Swaziland and resulted in diarrhea in 40,912 people but mortality data was unavailable (Effler *et al.,* 2001). In a review of literature on PubMed of *E. coli* O157:H7 publications in Africa about reservoir, transmission and diagnosis, Raji *et al.* (2003) reported 0157:H7 (VTEC) cases have been isolated from many sporadic cases of diarrhea, bloody diarrhea and HUS. Many cases of infection by pathogenic *E. coli* may exist in the population but may go undetected because of inadequate diagnostic tools at hospitals or inadequate prevalence research.

In an online search on PubMed (25 April 2012) about documented outbreaks or reports of pathogenic *E. coli* in Africa with the search word 'Africa EHEC', a total of five outbreaks investigation were carried out from 1988-2012 in five different countries. Two were recorded in Central Africa while one outbreak was recorded each in West Africa, East Africa, South Africa and none in Northern Africa. On prevalence and incidence investigations within the same period of 1988-2012 in Africa, a total result of 78 research publications were obtained, of which 66 investigations concerning pathogenic *E. coli* where conducted in 21 out of the 54 countries in Africa. There were 3 general reviews about pathogenic *E. coli* in Africa. Sixty one percent (33) of the countries in Africa did not publish any research on outbreak or prevalence that is present on PubMed with the search word 'EHEC Africa'. South Africa (12) recorded the highest number of published prevalence investigation followed by Nigeria (9) and Kenya (5). The lowest number of prevalence investigation was recorded in Central Africa. Ghana recorded only 2 published prevalence investigations. Even though not all the research publications get into PubMed, it is now used widely as a standard for

research publications. The number of reports or publications on pathogenic *E. coli* in Africa is relatively low compared with the same search word from other continents but substituting the name of the continents, for instance 'EHEC South America'=107, 'EHEC Australia'=156, 'EHEC Asia'=314, 'EHEC Europe'= 638, and North America ('EHEC Canada'=516, 'EHEC USA'=2173). Figure 7 shows results of number of publications of pathogenic *E. coli* per year from PubMed from 1988-2012 using the search word 'EHEC Africa'.

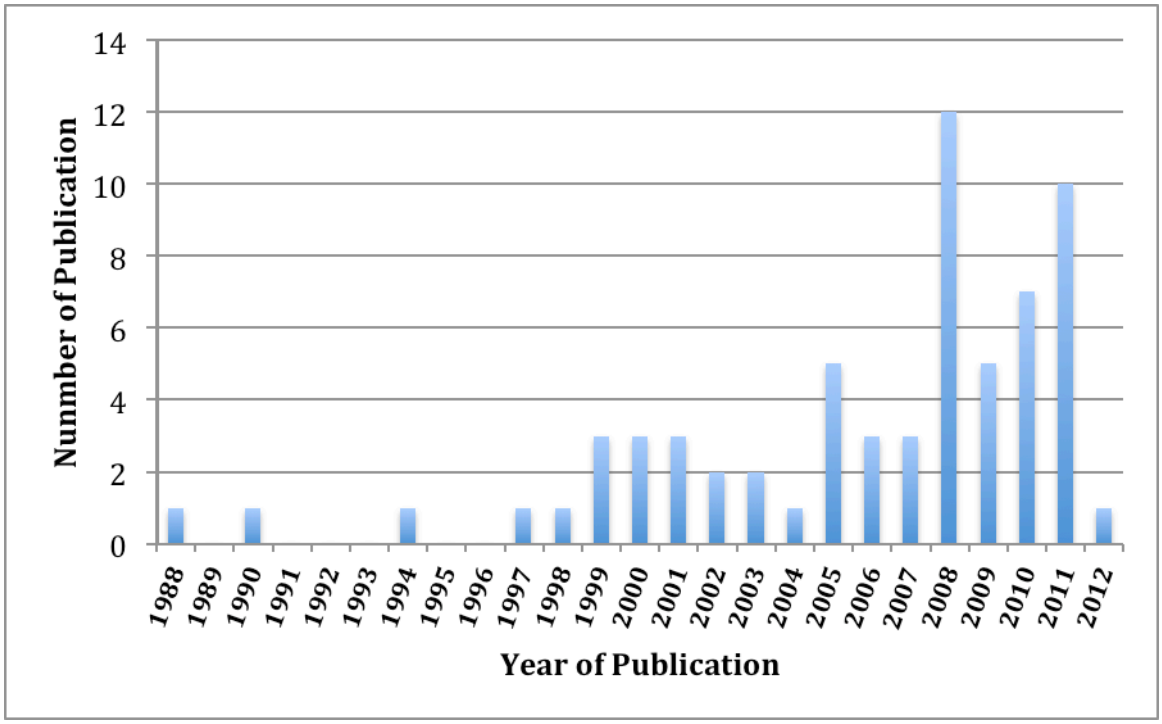

Figure 7. Number of publications from Africa on pathogenic *E. coli* using the search word 'EHEC Africa' on PubMed.

3.4.4 Epidemiology in Ghana

Currently, there is very little information on pathogenic *E. coli* in Ghana. Agbodaze *et al.* (1988) reported the existence of pathogenic *E. coli* as the cause of infantile diarrhea in Accra, the capital city since 1988. Addy *et al.* (2004) conducted a research about the prevalence of pathogenic *E. coli* in infants in

Kumasi, the second biggest city in Ghana and recommended that, EPEC may be considered an important diarrheagenic pathogen in the area. In a study conducted in the Southern parts of Ghana on the etiology of intestinal inflammation in children, Opintan *et al.* (2010) reported that EAEC was a common intestinal pathogen in Accra. In a microbial quality research assessment performed on informally marketed raw cow milk in Cape Coast, near Accra, Addo *et al.* (2011) reported that, there was no *E. coli* O157:H7 found in any of the 250 samples tested. As of now, there is no outbreak investigation data from Ghana probably because of the lack of facilities and materials as well as personnel that may respond to quick outbreak investigations.

Ghana is divided into two parts; the Southern zone and the Northern zone. All the prevalence investigations mentioned above about pathogenic *E. coli* were conducted in the Southern zone. There is currently no report about the prevalence of pathogenic *E. coli* in the Northern regions of Ghana. The Northern Regions have more limited access to portable water and health facilities than the Southern cities (DHS, 2003) and most communities lack portable drinking water. Moreover, there is more livestock in the North especially cattle and other ruminants, which are known as natural reservoir for pathogenic *E. coli,* than in the Southern zone. Most of the people especially in the rural areas rely on untreated water, which is sometimes shared by their animals. Even though the people in the Northern zone may be more at risk than those in the Southern zone there is not a single report about the risk posed by pathogenic *E. coli*. Furthermore, there is very little prevalence data on animals as sources of pathogenic *E. coli* infections. Recently, there exists only one research paper in southern Ghana on raw cow milk to check for *E. coli O157:H7* (Addo *et al.,* 2011). There is no prevalence data from

the Northern Regions on animals even though there is a high risk of its involvement in human infections in the area. There is also no report of APEC in Ghana although it may exist. In the third publication that constitutes this Thesis, we isolated and tested for the prevalence of Shiga toxin 1 and 2 producing *E. coli* from humans from the Northern Region of Ghana.

3.4.4.1 Roles in foodborne illness outbreaks, recalls and deaths

There is no documented outbreak as a result of pathogenic *E. coli* in Ghana, but there are reports that sought to isolate *E. coli* from food especially street foods (Mensah *et al*., 2002; Agbodaze *et al.,* 2005; Donkor *et al.,* 2007). All three studies cited above were only to isolate *E. coli* from food as an indication of fecal contamination, but were all done in the southern zone of Ghana. Currently, there is no concrete data of the fecal contamination of food in the Northern Regions of Ghana.

The Ghana Food and Drugs Board, the authority responsible for food regulations in Ghana do intercepted unwholesome foods occasionally (http://www.fdbghana.gov.gh/), but none was reported to contain pathogenic *E. coli* probably due to inadequate equipped laboratories to test for them. If foodborne illness is to be controlled, the government must invest in improving facilities and personnel to properly detect potential foodborne illnesses. There are no published or documented reports about death resulting from food due to the consumption of food containing pathogenic *E. coli*. This may be due to inadequate research to look for the pathogens in food. In the third publication of this Thesis, some street vended foods from the Northern Region were analyzed to isolate both non-pathogenic and pathogenic *E. coli*.

3.5 Pathogenicity and Virulence Factors

The acquisition of mobile genetic elements especially prophages and pathogenicity islands are known to be one of the major sources of acquisition of virulence factors by pathogenic *E. coli* (Schmidt, 2010). Another contributing factor of virulence in pathogenic *E. coli* is the locus of enterocyte effacement (LEE), which is implicated in the intimate adhesion of EHEC to the intestinal epithelial cells (Iyoda *et al.,* 2011). The evolution of these genomic regions through sporadic or frequent recombination of virulent genes as well as the acquisition or loss of chromosomal or plasmid DNA result in the creation of new pathogenic strains (Dobrindt, 2005). The T3SS is used by pathogenic *E. coli* once they adhere to the cells through their attaching and effacing (A/E) activity and cause intestinal inflammation of the cell by utilizing the T3SS to deliver effector proteins into their host cells (Sham *et al.,* 2011). Shiga-toxin (*stx*1 and *stx*2) productions are widely known to be a source of virulence in pathogenic *E. coli*. Shiga-toxin production strains harbor virulence-associated genes such as those described in Table 8. Plasmid acquisition such as pO157, through horizontal gene transfer is one of the main contributors to the virulence of pathogenic *E. coli* (Fratamico *et al.,* 2010; Wang *et al.,* 2011; Mallata *et al*., 2012). Virulence due to pathogenic plasmid acquisition by the rare strain *E. coli* 104:H4 was a major factor in the outbreak that hit Germany and other EU countries in 2011 (Rohde *et al.,* 2011; Künne *et al.,* 2012). Plasmids, prophages or bacteriophages are able to transfer virulent genes (Table 8) from pathogenic strains to non-pathogenic or less pathogenic strains, hence result in the pathogenicity in some commensal strains.

Table 8. Virulence-associated factors present in ExPEC

	Virulence factor
ADHESINS	
F10 *papA*	P fimbriae subunit variants
papC	*papACEFG* genes of fimbriae operon
papEFG	*papACEFG* genes of fimbriae operon
Sfa/foc	S or F1C fimbriae
focG	F1C fimbriae adhesion
Iha	Adhesion siderophore
fimH	Type 1 fimbriae
Tsh	Temperature sensitive hemagglutinin
Hra	Heat-resistant agglutinin
afa/draBC	Dr-binding adhesins
TOXIN	
hlyD	α-Hemolysin
Sat	Secreted autotransporter toxin
Pic	Serine protease
Vat	Vacuolating toxin
astA	Enteroaggregative *E. coli* toxin
cnf1	Cytotoxic necrotizing factor
SIDEROPHORES	
iroN	Salmochelin (siderophore) receptor
fyuA	Yersiniabactin (siderophore) receptor
ireA	Siderophore receptor
iutA	Aerobactin (siderophore) receptor
CAPSULE	
***kpsM* II**	*kpsM* II group 2 capsule
K1	K1 group 2 capsule variants
K2	K2 group 2capsule variants
K5	K5 group 2capsule variants
***kpsMT* III**	Group 3 capsule
MISCELLANEOUS	
Usp	Uropathogenic-specific protein
traT	Serum resistance-associated
ompT	Outer membrane protease T
Iss	Increase serum survival
H7 *fliC*	Flagellin variant
malX	Pathogenicity island marker

Source: Pitout, 2012

3.6 Genetic Diversity

The fact that the genetic diversity and genome plasticity of *E. coli* contributes to it pathogenicity, virulence and adaptation has been underestimated (Dobrindt, 2005). Within a group of clonal populations, there exists a diverse genetic make up as they continue to replicate their genetic materials to produce new generations. These new generations even though clonal can have several mutations in the subsequent generations and differ from the parental clonal population. In a study conducted by Caugant *et al.* (1981) on the genetic diversity of *E. coli* from a single individual (healthy almost throughout the study period) for 11 months, they obtained 550 clones and 53 distinct electrophoretic types from the same individual. Reports of *E. coli* from the same population from other ecological settings also showed great variety among the different strains (Jarvisa *et al.,* 2000; McCrea *et al.,* 2008; Ibekwe *et al.,* 2011). There are very few reports on the genetic diversity of *E. coli* isolated from humans, poultry and food from Africa. In the third publication of this thesis, we performed a pulse-field gel electrophoresis to determine the genetic diversity among and within the *E. coli* strains isolated from human, animal and food isolates.

3.6.1 Pulsed-field gel electrophoresis (PFGE)

This technique is now widely employed by epidemiologists to find out the genetic relationship between species of bacterial strains in several outbreaks (Lanier *et al.,* 2009; CDC, 2010; Januszkiewicz *et al.,* 2012). The technique involves digesting the DNA of bacteria with a specific enzyme at specific sites. The digested DNA is then run with an electrically pulsed field to separate the bands into sizes. The sizes are visualized under UV light to compare the pattern of the bands. Based on the size and relatedness of the bands, a software (BioNumerics) is used to

estimate strains that have the same patterns. The bands can also be seen and compared manually when there are only a few strains. Bands with the same patterns are considered to be the same strain and those with similar bands are designated closely related or possibly related.

3.6.2 Phylogenetic groups

Phylogenetic analysis has shown that *Escherichia coli* is composed of four main phylogenetic groups (A, B1, B2, and D) and that virulent extra-intestinal strains mainly belong to groups B2 and D (Clermont *et al.,* 2000). This analysis may be used to check a population of commensal *E. coli* and predict if the population contains individuals that can produce virulent strains and can result inin serious infections. The more virulent the strains in a population are, the higher the risk of causing illness. There are very few reports of phylogenetic analysis among *E. coli* populations from humans in Africa (Durez *et al.,* 2001; Okeke *et al.*, 2010) as well as animal and food isolates. There is no study in Ghana that employed phylogenetic analysis of *E. coli* as a mean of finding out how pathogenic *E. coli* populations are in humans, animals and foods. We performed a phylogenetic analysis of *E. coli* strains from humans, animals and foods from the Northern Region of Ghana in the third publication of this Thesis.

4. 0 Antibiotic Resistance

4.1 Antibiotics and Brief Overview of it Development

The first natural antibiotic substance historically recognized was pyocyanase, produced from the bacteria *Pseudemonas aeruginosa* in 1899 by two German physicians Rudolf Emmerich and Oscar Löw (Smith, 1946). The Scottish bacteriologist Alexander Fleming accidentally discovered the natural antibacterial agent, Penicillin, in 1928 while working with the bacteria *Staphylococcus aureus* (Fleming, 1929). He cultured *Staphylococcus aureus,* which was exposed to air and hence was contaminated by the mold, *Penicillium notatum.* He observed that the mold had bacteriocidal and bacteriolytic properties on pathogenic bacteria. However, it was not until 1940 that its therapeutic effect in human medicine was published (Flores *et al.* 1940). The first synthetic and commercially available antibacterial agent was Sulfonamide, which was developed in the 1930s by the German Bacteriologist Gerhard Domagk. The word antibiotic was first used in the 1942 by Selman Waksman to mean substances produced by microorganisms with a resultant antagonistic effect on other microorganisms. He discovered the antibiotic streptomycin from *Streptomyces griseus*. Streptomycin was the first line antibiotic used to cure tuberculosis and later many more antibiotics were discovered. Presently, antibiotic is used as a generic term to denote any class of organic molecule that inhibits or kills microbes by specific interactions with bacterial targets without any consideration of the source of the particular compound or class (Davies *et al.,* 2010). Nowadays, the words antibiotic and antimicrobial are used interchangeable to refer to antibacterial agents.

4.1.1 The genesis of antibiotic resistance

Antibiotic resistance genes seem to have developed in nature with the evolution of bacteria several years before the human acquaintance with resistance determinants in bacteria and its environments. In a recent study conducted by Bhullar *et al.* (2012) in a Mexican Cave that has been isolated for over 4 million years, they reported that bacteria isolated from those regions were highly resistant to commercial antibiotics and possessed several antimicrobial resistance genes, even though these bacteria had not previously come into contact with any antibiotic pressure. This is typical evidence that resistance genes have evolved with us since antiquity. Even before the large-scale production of penicillin antibiotic for clinical use, there was a report of a substance that inactivated the growth-inhibiting property of penicillin produced by *E. coli* (Abraham *et al.,* 1940), and by the middle of 1940, there were more reports of antibiotics resistance (Waksman *et al.,* 1945; Selbie, 1946; Miller *et al*., 1946). The widespread and indiscriminate use of antibiotics in their early years without prescription was observed to be a major factor in the early development of resistance to antibiotics (Thomson, 1952). Since the discovery of the enzyme that inactivates penicillin (Penicillinase) in the 1940 the world has been grappling with new enzymes and mechanisms used by bacteria to confer resistance to newly developed antibiotics. Although antibiotics have saved millions of lives during the past years, in recent times the problem of antimicrobial resistance has become a major threat to the world. Some experts even fear that, we are returning to the pre-antibiotic era.

A typical example of the implication of the frequent use and selective pressure of antibiotics in the selection of antimicrobial resistant strains can be

observed from the latest use of a fifth generation cephalosporin drug, Ceftraroline. The USFDA approved this antibiotic in the 2010 for the treatment of bacterial infections, but a survey conducted in the USA by the Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) indicated that *Staphylococcus aureus* showed 98.4 % susceptibility and the enterobacteriaceae also showed a remarkable susceptibility against ceftraroline (Flamm *et al.,* 2012). This situation is similar to the introduction of penicillin, which was the most potent antibiotic in the 1940s but currently, it is one of the least potent or most resistant to bacteria. Presently, ceftraroline is not widely and frequently prescribed so it seems to be the 'magic bullet' of the moment. Perhaps the bacteria are now developing their defenses by acquiring new genes or recombining their genes to develop another resistance mechanism to this newly introduced drug. Who knows? Let us leave the answer for the near future. It is obvious that the frequent misuse of antibiotics has actually deepened the antimicrobial resistance problem in the world as a result of selective pressure on the microorganism. However, antimicrobial resistant organisms have also been found in the environment, hospitals or communities where there is no selective or direct antimicrobial pressure (Coombs *et al.,* 2011; Brennan *et al.,* 2012). Antimicrobial resistance can also be caused or enhanced by agents, which contain antimicrobial properties (disinfectants, detergents, biocides etc.) outside the body (Sidhu *et al.,* 2002; Aiello *et al.,* 2003) and later enter into humans mainly because of unhygienic conditions. However, a study in a Bolivian Community on commensal *E. coli* reported a high prevalence of resistance to antibiotics in the populace was not related to heavy consumption of antimicrobials (Bartoloni *et al.,* 2004).

In animals, antibiotics are not only used for therapy and prevention of bacterial infections, but are also added to animal feed to act as growth promoters (van den Bogaard *et al.,* 2000). One of the contributing factors of antimicrobial resistance observed especially in Europe, which is still unclear in the USA (till 2011) is the scientific evidence that the frequent use of antimicrobial agents in the production of animals had contributed to the build up of resistance in animals, which was then passed on to humans (Van den Bogaard *et al.,* 1996; Marshal *et al.*, 2011). The Swedish banned all food animal promoting antibiotics in 1986 and the EU has banned the use of avoparcin as a growth promoter in livestock production since 1997 and later bactricin, spiramycin, tylosin and virginiamycin in 1999 (Casewell *et al.,* 2003). Since then there have been reports of a decrease in multidrug resistance in both animals and humans (Pantosti *et al.,* 1999; Van den Bogaard *et al.,* 1999; van den Bogaard *et al.,* 2000). Due to these encouraging results, the EU banned the feeding of all antibiotics and related drugs to livestock for growth promoting purposes since 2006.

4.2. A Brief Status of The Global Burden of Antibiotic Resistance

According to the WHO, "the reasons why antimicrobial resistance is of global concern are that; it kills, it hampers the control of infectious diseases, it threatens a return to the pre-antibiotic era, it increases the costs of health care, it jeopardizes health-care gains to society, it threatens health security, and damages trade and economies." The burden and the widespread of antimicrobial resistance has become pandemic and remains one of the medicare challenges of this century hence strategies must developed to tackled it internationally through collaborations (Cars *et al.,* 2011). In advanced countries there are constant

surveys on antimicrobial resistance but in resource-limited or developing countries very few articles report antimicrobial resistance (Allegranzi *et al.,* 2011). The problem is also exacerbated due to decrease in research into new antimicrobials, decrease in the development of new antibiotics and increasing rates of antibiotic resistance. Reports from the Study for Monitoring Antimicrobial Resistance Trends (SMART) indicated that since 2004-2007, there has been increasing antimicrobial resistance worldwide in gram-negative bacteria isolated from intra-abdominal infections (Rossi *et al.,* 2006; Baquero *et al.,* 2009; Hawser *et al.,* 2009).

4.2.1 *Europe*

Reports from the European Antimicrobial Resistance Surveillance Network (EARS-Net) from 2002 to 2009 revealed that, the antimicrobial susceptibility test from 198 laboratories in European Countries had an upsurge of bloodstream infections caused by *E. coli* (71%) during the study period and that there is an increasing burden of disease caused by *E. coli* (Gagliotti *et al.,* 2011). A report in 2007 estamated the mortality and hospital associated infections with resistant *Staphylococcus aureus* and *E. coli* bacteremia as well as the estimated burden of antibiotic resistance in Europe (de Kraker *et al*. 2011). They reported Methicilin Resistant *Staphylococcus aureus* (MRSA) bloodstream infections were associated with 5,503 excess deaths and 255,683 excess hospital days in the participating countries, whereas third-generation cephalosporin-resistant *E. coli* bloodstream infections were associated with 2,712 excess deaths and 120,065 extra hospital days. They concluded that the total costs attributable to excess hospital stays for MRSA and third-generation cephalosporin-resistant *E. coli* bloodstream infections were 44.0 and 18.1 million Euros (63.1 and 29.7 US dollars), respectively.

4.2.2 United States of America

In the USA, Maragakis *et al.* (2008) reported that patients with infections due to antimicrobial resistant (*Staphylococcus aureus, enterococci* and Gram-negative bacilli) organisms have higher costs (US $6,000-30,000) than do patients with infections due to antimicrobial-susceptible organisms. He further reported that, the difference in cost is even greater when patients infected with antimicrobial-resistant organisms are compared with patients without infection. Although both studies (USA and Europe above) were species specific except that the USA study was a bit broader in terms of species and among other factors, the extra cost as a result of antimicrobial resistance in Europe seems to be higher than that of USA. The cost burdens observed in Europe and USA might have resulted in reduced death rate of the population because of the relatively higher income levels but the same cost burden in Africa and other developing countries would have resulted in several deaths since most of the patients may not have the income to afford the extra cost burden as a result of antimicrobial resistance.

4.2.3 Asia

There is no general report on the antimicrobial resistance burden on the whole Asian population like the EU and USA, but data are normally estimated based on some reports from the individual countries. Generally, it has been reported that the antimicrobial resistance burden in Asia is high (Jean *et al.,* 2011). In a recent epidemiological survey conducted in the Asian Pacific Region, Hsueh *et al.* (2011) reported that nearly half of *E. coli* urinary isolates were resistant to levofloxacin or ciprofloxacin and ≥30% were resistant to third-generation cephalosporins. They further reported that prevalence of ESBL-producing urinary *E. coli* was highest in India (60%), followed by Hong Kong

(48%) and Singapore (33%). Other recent reports of antimicrobial resistance in the continent are: Thailand (Nikerson *et al.,* 2011), Saudi Arabia (Saeed *et al.,* 2010), China (Zhao *et al.,* 2012) and Japan (Harada *et al.,* 2012). A global report on the *In vitro* susceptibilities of aerobic and facultatively anaerobic Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide in 2004 and 2005, observed that there is generally higher rates of antimicrobial resistance in gram negative bacterial infection isolates from intra-abdominal infections in the Asia/Pacific region compared to the rest of the world for two consecutive years (Rossi *et al.,* 2006; Baquero *et al.,* 2009).

4.2.4 Latin America

Latin American countries, like other developing countries do not have precise estimate of the burden of antimicrobial resistance. According to a recent report from Mexico on the situation of resistant *E. coli* from bronchial secretions, urine, central catheter, blood, and infected wounds, in a Hospital, Murillo-Llanes *et al*. (2012) reported a very high rate of resistance of *E. coli* to commonly used antimicrobials. A similar report was also recorded in uropathogenic *E. coli* (UPEC) from patients in Mexico (Molina-lopez *et al.,* 2012). Increasing resistance in commensal *E. coli* in Bolivia and Peru has been reported (Bartoloni *et al.,* 2008). There are reports of very high ESBL resistant *E. coli* by Villegas *et al.* (2011) from 10 Latin American countries including Chile (Garcia *et al.,* 2011), and Colombia (Gaitán *et al.,* 2009). In a recent report on *E. coli* from 11 Latin American countries, out of 1366 *E. coli* isolates, 323 (23·6%) were ESBL producing (Hawser *et al.,* 2012).

4.2.5 Australia and New Zealand

There have been increased reports of multiple resistance and ESBL producing *E. coli* in Australia since 2008 to 2009 (http://www.agargroup.org/surveys). Australia has structured antimicrobial surveillance similar to the EU and USA, but currently there is no estimated burden of antimicrobial resistance. There are also published reports about the increasing spread of antimicrobial resistance determinants in *E. coli* and *S. aureus* in Australia (Gündoğdu *et al.*, 2011; Holmes *et al.,* 2011; Ramos *et al.,* 2011), which directly or indirectly has a burden on patients and the government. In New Zealand, there have been reports of antimicrobial resistance in *E. coli* producing ESBL (Briggs *et al.,* 2005) and among travelers to the Indian subcontinent producing CTX-M-15 (Freeman *et al.,* 2008).

4.2.6 Africa

The issue of antimicrobial resistance in Africa is a worrying one because unlike other countries or continents, the precise burden is not known, but the severe effect of the phenomenon can be observed in the populations who are plagued by infections caused by bacteria (Okeke *et al.,* 2007). The true burden of antimicrobial resistance in not known due to unavailability of published research works among a long list of other factors. This problem is further complicated by the low illiteracy rates in African countries.

In Africa, even though the rate of research into prevalence and resistance mechanisms is very low, there are few reports that showed that there is increased rate of antimicrobial resistance. Virulence resistant determinants in non-typhoidal *Salmonella* isolates have been documented recently in West African countries such as Gambia and Senegal (Dione *et al.,* 2011), Ghana (Gross *et al.,*

2011), Mali (Boisramé-Gastrin *et al.*, 2011), Nigeria (Akinyemi *et al.,* 2011). There have also been reports of high resistance of both commensal and pathogenic *E. coli* to antimicrobials in Nigeria (Okesola *et al.,* 2011; Lamikanra *et al.,* 2011), Ghana (Namboori *et al.*, 2011), and Senegal (Ruppé *et al.,* 2009).

In other parts of Africa, there have been reports of increased antimicrobial resistant determinants from *Salmonella* in Algeria (Naas *et al.,* 2011), Morocco (Ohmani *et al.,* 2010), South Africa (Smith *et al.,* 2010), Kenya (Mengo *et al.,* 2010), Ethiopia (Beyene *et al*., 2011), Tunisia, Cameroon and Senegal (Hendriksen *et al.,* 2012). Increasing resistance of both commensal and pathogenic *E. coli* to antimicrobials have been reported in Kenya (Onyango *et al.,* 2009), Botswana (Magwira *et al*., 2008), South Africa (Abong'o *et al.,* 2009) and Rwanda (Muvunyi *et al.,* 2011).

4.3 Overview of Antibiotic Resistance in Ghana

The antimicrobial resistance burden of Ghana is not known, but the major problems in the fight against antimicrobial resistance may include (i) extremely low knowledge on microorganisms and antibiotics (ii) inadequate documentation of prescriptions in hospitals (iii) purchase of antibiotics without a prescription (iv) sales of antibiotics by untrained pharmacists (v) inadequate diagnosis of diseases by healthcare professionals before issuing antibiotics (vi) inability of patients to complete the prescribed dose of antibiotics (vii) inadequate laboratories for performing antimicrobial susceptibility test (viii) the culture of self-medication (ix) availability of fake antibiotics in the market (x) inadequate antibiotic stewardship programs in hospitals (xi) inadequate research on resistance in both community and hospital acquired infections (xii) inadequate

surveillance and monitoring of antimicrobial resistance (xiii) inadequate knowledge of farmers on antibiotics resulting in farmers giving any antibiotics to their animals (xiv) inadequate public education on antibiotics (xv) non existent antibiotics policies by national governments and (xvi) laxity in the enforcement of antibiotic policies.

Even though there is not a constant antimicrobial resistance surveillance program that covers the whole of Ghana, publications on antimicrobial resistance in Ghana about *Salmonella* exist (Newman, 1996; Sackey *et al.,* 2001; Mills-Robertson *et al.,* 2002 and 2003; Djie-Maletz *et al.,* 2008; Schwarz *et al.,* 2011; Gross *et al.,* 2011). A recent study conducted by Newman *et al.* (2011) in seven of the ten regions in Ghana, multidrug-resistant strains of *Staphylococcus aureus*, *S*. Typhi, and non-typhoidal *Salmonella* had a high MIC to cefuroxime, gentamicin and ciprofloxacin. There are few reports about the susceptibility level of *E. coli* isolated from humans in Ghana (Ohene, 1997; Sackey *et al.,* 2011; Newman *et al.,* 2002; Adjei *et al.,* 2004; Djie-Maletz *et al.,* 2008; Namboodiri *et al.,* 2011). The most recent study on the prevalence of antimicrobial resistance in animals reported a high rate of resistance of *E. coli* to commonly used antibiotics (Donkor *et al.,* 2012). Almost all these research works were done in the Southern parts of Ghana leaving the Northern parts of Ghana with little information about antimicrobial resistance surveillance. Actually, there is no major information about the presence of resistant determinants and the real status of antimicrobial resistance in the Northern regions of Ghana. In the second and third publications of this thesis, we performed antimicrobial susceptibility test on all the strains isolated from humans, animals, and street foods in order to get a fair view of the current state of antimicrobial resistance in *Salmonella* and *E. coli* in the Region.

4. 4 Mechanisms of Antibiotic Resistance

The types of mechanisms of resistance developed by bacteria against antibiotics are most often dependent on the classes of antibiotic exposed to them. The main mechanisms are:

(a) Production of enzymes to hydrolyze essential bonds of the antibiotics, which render it unable to get to the target site and hence inactivate it from performing its function in the bacteria. For instance the production of beta lactamases by most bacteria to hydrolyze the beta lactam bonds in the beta lactams antibiotics such as penicillin, ampicillin, cephalosporins.

(b) Target modification, which involves the bacteria producing mutations in the targeted site of attachment of the antibiotic in the bacteria and hence make the antibiotics unable to exert their effects on the bacteria. For instance aminoglycoside drugs such as kanamycin, streptomycin and amikacin are resisted by targeted mutations produced by bacteria on the 30S ribosome (16S rRNA methylase). They are also produced against some quinolone or fluoroquinolone drugs such as ciprofloxacin, levoxacin by the mutation of the *gyrA, parC* or topoisomerase targets involved in DNA replication, to evade antibiotics.

(c) Actions of efflux pumps, which are enzymes produced by bacteria to eject the antibiotics from the bacteria and hence reducing the concentration of the antibiotics in the bacteria to non-lethal doses that will not have effects on the bacteria.

(d) Alteration of the membrane proteins such as porins to prevent the antibiotics from entering the bacterial cell.

4.4.1 Resistome of E. coli

The resistome of *E. coli* is very important in the control of antimicrobial resistance due to the fact that the organism can be found naturally in the gastrointestinal tracts of both humans and animals. Furthermore, its plasticity makes it a reservoir for the acquisition of antimicrobial resistance determinants from other species or their environment, which make them pathogenic. Yet, the main concern remains the possible transfer of the resistant determinants through mobile genetic elements (conjugative plasmids or prophages) and other intra-genetic elements such as integrons, transposons, efflux pumps etc. to other pathogenic or commensal species through vertical or horizontal gene transfer or to other genera through horizontal gene transfer in the gastrointestinal tract of it hosts. An insight on how these resistance mechanisms develop and render antibiotics incapable of performing their target functions may help to develop new effective and efficient antibiotics (Wright , 2007).

4.4.2 Plasmid mediated antimicrobial resistance in E. coli

Plasmid mediated resistance was first described in the in the 1950s by Josua Lederberg to mean all extra-chromosomal hereditary determinants (Lederberg, 1952). These hereditary determinants have the ability of replicating independently from the chromosomal DNA. The implications of plasmids in the transfer of resistance were not clear until in 1961, when Watanabe and Fukasawa finally proved that plasmids were involved in the transfer of multiple drug resistance determinants of genes encoding for streptomycin, chloramphenicol, tetracycline and sulfonamide and that they were successfully transferred from

Shigellae to *E. coli*, *S.* Typhimurium, and *S*. Enteritidis by conjugation (Watanabe *et al.,* 1961). Since then a lot of research has found resistance determinants carried on plasmids from *E. coli* and others in the family Enterobacteriaceae and *Stapylococcus aureus* (Salzman *et al.,* 1967; Peyru *et al.,* 1969; San-Millan *et al.,* 2009; Fratamico *et al.,* 2010; Wang *et al.,* 2011, Mallata *et al.,* 2012 Künne *et al.,* 2012).

4.4.2.1 Plasmid mediated TEM beta lactamase

The first plasmid mediated beta lactamase (TEM) enzyme resistance was first described in the mid 1960s (Datta *et al.,* 1965) and was commonly found later in the 1970s to be the main mechanism for the inactivation of beta lactam antibiotics in gram-negative bacteria (Kontomichalou *et al.,* 1974; Heffron *et al.,* 1975). The enzyme was designated TEM because of the name of the first patient from which it was isolated, named Temoniera from Greece (Medeiros, 1984). The TEM enzymes especially TEM-1 are widely distributed in most strains of *E. coli* and other enterobacteriaceae and frequently inactive against ampicillin (Bradford, 2001).

In Africa, although the presence of TEM-1 and other TEM enzymes might be very high due to the high level of auto-medication especially with the commonly used antibiotics such penicillin and ampicillin, there are only a few reports on the prevalence of TEM in the population. The first reports of TEM-1 in Africa were probably found in the 1980s in enterobacteriaceae in Senegal (Philippo *et al.,* 1984; Shaokat *et al.,* 1987), but later were reported in a number of studies across the continent; Algeria (Rahal *et al.,* 1994), South Africa (Shanahan *et al.,* 1995), Kenya (Kiriuki *et al.,* 1996), Nigeria (Soge *et al.,* 2006), and Burkina faso (Zeba *et al.,* 2007).

In Ghana, the presence of the TEM enzyme has not been studied yet in the Northern Region of Ghana or the whole Ghana, but the high rates of resistance to ampicillin and common or older beta lactam antibiotics (Mills-Robertson *et al.,* 2003; Newman *et al.,* 2011), suggest that the enzyme might be common. In the third publication of this Thesis, we determined the presence of TEM in some of our strains.

4.4.2.2 Extended-spectrum beta lactamase (ESBLs)

ESLBs are generally acquired by horizontal gene transfer and confer resistance to oxyimino-cephalosporins, some being mutant derivatives of established plasmid-mediated beta lactamase (eg TEM/SHV) or mobilized from the environment (eg CTX-M) (Hawkey *et al.,* 2009), but are inhibited by clavulanate. They can be chromosomally encoded or mediated by plasmids and transposons (Medeiros, 1984). The first plasmid-mediated ESLB was described in Germany in 1983 (Ho *et al.*, 2010). The CTX-M ESBLs were first described in 1989 (Davies *et al.,* 2010). Currently the ESBLs producing strains, which encode for the CTX-M genes have been found to be spread globally with the CTX-M-15 plasmid being the most common (Naseer *et al.,* 2011).

In Africa, CTX-M-15 has been studied and reported in only a few countries: Nigeria (Soge *et al*., 2006), Algeria (Naas *et al.,* 2001), Senegal (Ruppé *et al.,* 2009), Cameroon (Gangoue-Pieboji *et al*., 2005), Tanzania (Bloomberg *et al.,* 2005), Malawi (Gray *et al.,* 2006), Kenya (Kariuki *et al.,* 2007), South Africa (Usha *et al.,* 2008), Morocco (Barguigua *et al.,* 2011), and Mali (Boisramé-Gastrin *et al.,* 2011). Currently, there is no report of CTX-M from Ghana.

4.4.2.3 Plasmid-mediated quinolone resistance

Although quinolone resistance genes are commonly found on chromosomal mutation, plasmid mediated resistance has been discovered and the gene responsible for plasmid-mediated quinolone resistance is called *qnr* (Wang *et al.,* 2004). These genes normally cause reduced susceptibility in quinolone antibiotics. The first plasmid-mediated quinolone resistance was confirmed by Martínez-Martínez *et al*. (1998) in a *Klebsiella pneumonia* strain from Birmingham, Alabama, USA, which produced an increase in the MIC in other enterobacteriaceae and in *Pseudomonas aeruginosa* after the plasmid-mediated gene was successfully conjugated into them. Ever since, the plasmid-mediated gene (*qnr*) has been described in a number of studies in *E. coli* and other enterobacteriaceae as well as other gram-negative bacteria (Wang *et al.,* 2004; Guitierrez *et al.,* 2009; Jeong *et al.,* 2011; Riveros *et al*., 2012).

There are very few studies about plasmid-mediated quinolone resistance in Africa: Egypt (Ahmed et al., 2009), South Africa (Govender *et al.,* 2009), Tunisia (Dahmen *et al.,* 2010), and Nigeria (Ogbolu *et al.,* 2011). In Ghana, there is currently only one report of plasmid-mediated quinolone resistance from the capital city, Accra (Namboodiri *et al.,* 2011).

4.4.2.4 Integron-mediated antimicrobial resistance

Integron genetic capture system that captures gene cassette units and converts them to functional genes by ensuring their correct expression. Integron was first described by Stokes and Hall in 1987 (Stokes *et al.,* 1989). A typical class 1 integron consists of an integrase gene (*IntI*) with a promoter for the cassettes found the 3´end of the integrase. The insertion site (*attI*), and the gene cassettes, which have a short base sequence called the 59-base element (*attC* site), which

facilitate the integration of other gene cassettes by recombining with the insertion site (*attI*). The variable region between the 5´and the 3´ ends may or not contain antibiotic resistance gene cassettes. At the 3' end of the cassettes, there is a conserved region, *sulA and qacEΔ1* genes that encode for sulfonamide and quaternary compounds respectively (Radstrom *et al.,* 1991; Paulsen *et al.,* 1993). Figure 8 shows a sketch of typical Class 1 integron. Integrons are ubiquitous and are noted in many Gram-negative bacteria and are mainly borne on mobile genetic elements, plasmids, and transposons, which promote their spread within bacterial communities (Stalder *et al.,* 2012). Integron can also be located in the chromosome of bacteria (Cambray *et al.,* 2012). The gene cassettes encode for specific resistance genes to different classes of antibiotics. There is widespread integron encoded antimicrobial resistance in *E. coli* (Fluit *et al.,* 1999; Chang *et al*., 2000; Lee *et al.,* 2001; Sunde *et al.,* 2005; Najibi *et al.,* 2012).

In Africa, only a few reports have been documented regarding integrons in some countries: South Africa (Adrian *et al.,* 2000), Senegal (Gamassa *et al.,* 2004), Tunisia (Abbassi *et al.,* 2008), Nigeria (Chah *et al.,* 2010), and Egypt (Ahmed *et al.,* 2011). In Ghana, there are very few studies about integron-mediated antimicrobial resistance (Opintan *et al.,* 2008; Thompson *et al.,* 2011), but not reported in *E. coli* isolates.

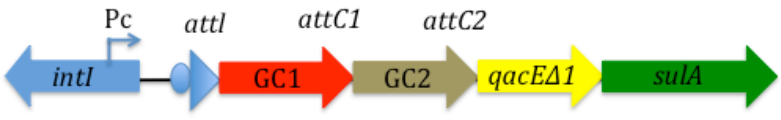

Figure 8. A typical Class 1 integron. The *intI* encodes for integrase, Pc is the promoter region of the gene cassettes GC1 and GC2, *attI* is the insertion site and *qacEΔ1* and *sulA* are conserved regions. *Source: Modified from Stalder et al., 2012*

“Reality leaves a lot to the imagination.”
—John Lennon

Objectives and Justification

The general objectives of this work is to determine the status of microbial food safety and antimicrobial resistance mechanisms of *E. coli* and *Salmonella* isolated from humans, animals and street foods from the Northern Region of Ghana.

The hygienic practices of most of the population in the African continent are generally low and Ghana is no exception. The Northern region is one of the poorest Regions in Ghana in terms of health delivery and infrastructure. Most of the diseases that occur in Ghana and its Northern Region in particular are foodborne related and more often than not, are preventable, but for the low hygienic practices of the people as a result of inadequate public education and believes.

Furthermore, the level of education concerning the proper use of antibiotics is also very low. This problem is compounded by the increasing number of people who rely on self-medication with antibiotics for diseases that are not caused by bacterial infections. Antibiotics are commonly sold to the people without prescriptions and when a patient is lucky enough to consult a physician; antibiotic susceptibility is rarely performed on suspected microoganims before issuing the patients with antibiotics. These practices are globally known to have a serious repercussion on the fight against antimicrobial resistance. Moreover, there are inadequate antibiotics stewardship programs to help monitor and survey the level of antimicrobial resistance to assist in curbing the problem of multidrug resistance in antibiotic therapy.

The specific objective of this Ph.D. Thesis is to isolate, identify and molecularly characterize *E. coli* and *Salmonella* obtained from humans, animals and street foods from the Northern Region of Ghana. The results of this work, we hope will contribute immensely to elucidating the prevalence of antimicrobial

resistance and the main mechanisms of resistance employed by *E. coli* and *Salmonella,* as well as their contributions to antimicrobial resistance in other pathogens in Northern Region and Ghana as a whole.

“Equipped with his five senses, man explores the universe around him and calls the adventure Science.”
—Edwin Powell Hubble

Publications

Publications

This Thesis is composed of 3 manuscripts that are on various stages of being published. The first publication has been accepted and the second one is under revision in the Journal of Infection in Developing Countries. The third manuscript is submitted to PLoS Neglected Tropical diseases.

1) Microbial Food Safety in Ghana: A Meta-Analysis

Microbial Food Safety in Ghana: A Meta-Analysis

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Key words*: Meta-analysis; Microbial Food Safety; Public Health; Ghana*

RUNNING TITLE: Analysis of the Food Safety publications from Ghana.

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Abstract

Background

Food safety is a crucial factor to elicit the development of less favored countries worldwide. Here, we present a Meta-analysis of microbiological food safety publications from Ghana.

Methodology

The search words "Ghana food safety", "Ghana food research", and "Ghana food bacteria" were used to search for microbiological food safety researches in PubMed with related abstracts or title. We obtained 183 research articles of which, 11 articles relating to microbial food safety were selected after excluding those on ready-to eat microbial fermented foods, waterborne microorganisms and those without abstract. The time frame considered was 1997-2009. The criteria used were based on some methodological soundness developed in the medical field with some modifications.

Results

The methodological quality of the articles was generally sound, but most of them did not give directions for future research. Most did not state possible directions for future studies. The most predominant bacteria in Ghanaian foods are *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp. and *Escherichia* spp., which were found to be present in 65%, 50%, 46% and 38%, respectively, of all the food samples considered in the articles analysed in this work. The most contaminated food samples were macaroni, salad, and milk.

Conclusion

The rate of microbiological food safety research in Ghana calls for concern. We found that the trend in the publication of microbial food safety articles is abysmal. Hence an

important effort in research on food safety is needed in Ghana and other developing countries to help curb the incidence of preventable foodborne diseases.

Introduction

According to World Health Organization, about 1.8 million deaths are recorded annually worldwide as a result of diarrhoeal diseases and most of these cases are attributed to contaminated food and water. Although the vast majority of cases are mild, a significant number of deaths do occur and high levels of acute infections and chronic sequelae lead to billions of dollars in medical costs and lost of productivity [1] as well as frequent recalls. The problem of food safety is not only a problem in the developing countries, but also the developed countries, which have an advance food chain monitoring system. Foodborne diseases will continue to be a matter of major concern around the world in the foreseeable future, despite some important national success of reducing the levels of certain pathogens in foods resulting from better farm practices, food regulations etc. [2]. In the United States foodborne diseases have been estimated to cause 76 million illnesses, 323,000 hospitalizations, and over 5,000 deaths annually [3]. The production of food is very complex and the various and complex points of critical control of pathogens leave many routes of exposure from farm to fork food production continuum [4]. These problems are worst in the developing countries where the issues of food safety and security are major hindrances to their development. Minimizing the consumption of unsafe food, therefore, may serve as a key to ensuring good health of the population and plays a vital role in the economic progress of developing countries considering budget spent by both governments and development partners annually in confronting these problems. Diarrhea is the most common illness occurring in international travelers to developing tropical and semitropical regions [5] and the outbreak of the lethal *Escherichia coli* in Europe in 2011 highlighted the shortcoming of our knowledge on the basic principles of the evolutionary trends of new pathogens [6].

Meta-analysis critically combines the results of different scientific studies that address a set of related research hypotheses. This serves as a reference point for important information for food safety and policy decision makers. Here we used meta-analysis to analyze the microbial food safety articles about Ghana on PubMed, to give a general view of microbial food safety research in Ghana to guide future research and to serve as information base for policy makers in formulating food safety policies.

Materials and methods

Criteria for selection food safety articles

In this analysis, articles relating to microbial food safety in Ghana from both local and International Journals were searched from PubMed and meta-analyzed to assess the state of microbial food safety in Ghana. Three search words were used: "Ghana Food Safety", "Ghana Food Research" and "Ghana Food Bacteria". Twenty articles were recovered when the search word "Ghana Food Safety" was used. Eighty-three articles were recovered when the search word "Ghana Food Bacteria" was used whiles hundred and eighty-three articles were recovered when the search word "Ghana Food Research" was used. All the microbial food safety articles were selected based on their titles and further critically evaluated after reading the abstracts of those relating to microbial food safety. The articles on ready-to-eat microbial fermented foods and those without abstract were excluded from this work. We did not have access to those without abstract for initial screening. Moreover those without abstract were few (2) and were old publications. Those publications concerning waterborne diseases were also excluded from this analysis. The whole articles (11) were perused and examined based on a quality assessment checklist that was created for the assessment and validation of methodological soundness [7] with modifications for applications to food safety research [4]. The time frame considered was 1997-2009. The selected articles were grouped into three categories based on whether: (1) the articles were only to address a specific intervention (2) the articles addressed multiple interventions or (3) the article is general with specific interventions on food safety. Three major sub-classifications were made based on (i) the type of pathogen reported in the articles (ii) the food production sector or the stage at which the food was considered for the research and (iii) the commodity group under which the articles sought to research.

The three sub-classifications were sub-divided based on other seventeen sub-criteria (Table 1) [4]. The various food items involved in the eleven articles were identified and associated with the respective microbes that were isolated with them and further classified according to their frequency of occurrence in the food items. The same genera or species of microorganisms found in a food item in the same article were mentioned as a single microorganism. For instance 20 isolates of *E. coli* found in the same food by name and the same article were considered as one *E. coli* isolate (Table 1). The searches were conducted in August 2009. Complete citations of the articles used in the meta-analysis are listed in the appendix.

Results

Sources and location of articles

Eleven articles were selected for this work based on the exclusion criteria mentioned. The articles involved were from the following Journals: Acta Tropica (3), Ghana Medical Journal (2), International Journal of Microbiology (2), East African Medical Journal (1), Journal of Urban Health (1), Archives of Environmental Contamination and Toxicology (1), and Bulletin of the World Health Organization (1). Food safety publications were observed to concentrate on the Southern half of the country and majority of the publications were done in Accra and its environ (Fig. 1).

Microbial food safety interventions and methodological quality of articles

One article was published in 1997 whiles ten were published from 2000 to 2008. Three articles were published concerning a single bacterium specie whiles five articles concerned multiple bacteria species. Three articles did not specify the microorganism involved (Table 1). At the food production sector, an article each took into consideration processing and storage. Four articles took into consideration items at the farm level. Majority (8) of the articles were conducted at the post-processing stage while six researched were performed on street food. Two articles were focused on consumers whiles four articles were related to food vendors (Table 1). At the commodity group level, none of the articles researched on Aquaculture, Seafood or Shellfish. One article each researched on vegetables, chicken and pork. One article each researched on milk products and mixed products (different food products) whiles three articles focused on ruminant meat (Table l).

All the articles analyzed except one were focused on the intervention question for which they were carried-out and only an article out of the eleven articles did not describe the method of locating evidence. Only eight of the articles used explicit

criteria to select studies. The methodological qualities of the primary study of all the articles were critically appraised. The quantitative summaries of interventions effectiveness among the studies were observed in ten of the articles. The possible reasons for differences between studies were also given in ten of the articles. About the generalizability of the results to the target groups, eight of the articles generalized the result to the target group. Only two of the articles proposed directions for future research whiles three of the articles did not mention the pathogen of interest.

Types of bacterial isolates and frequency in food

One hundred and five isolates were obtained from a total of twenty-six food samples involved in the articles meta-analyzed (Table 1). Out of the one hundred and five bacterial species isolates, only five (5%) were gram-positive pathogenic bacteria, which were found in four food samples. The vast majority of the bacterial species isolates (95%) which were found in almost all the samples were Enterobacteriaceae (Table 2). It is worth mentioning that most of the isolates (83%) were isolated and identified in a major research carried out in Accra [8], in which they identified 91% of their isolates to the species level. The other 10 publications only isolated and identified 17% of isolates in this work. Of these 10 publications, only 23% of the isolates were identified to the species level of which *E. coli* is the most easily identifiable specie. *Enterobacter* spp. were found in seventeen (65%) out of the twenty-six samples whiles thirteen (50%) food samples contained *Citrobacter* spp. *Klebsiella* spp. and *Escherichia* spp. were found in twelve (46%) and ten (39%) samples respectively with six (23%) food samples containing *Pseudomonas* spp. *Serratia* spp. and *Chryseomonas* spp. were recorded in five (19%) and four (15%) samples respectively. *Staphylococcus* spp. was observed in three (12%) of the samples. *Acinobacter* spp., *Proteus* spp., *Shigella* spp., *and Salmonella* spp. were isolated from two (8%) food samples. *Yersina*

spp., *Erwina* spp., *Bacillus* spp., *Kluyvera* spp., *Mycobacteria* spp., *and Campylobacter* spp., were found in only one (4%) of the food samples (Fig 2). *Salmonella typhi* was also isolated from six venders in an article, which sought to determine the salmonella carrier status of venders.

Food types and associated bacterial species

Out of the twenty-six samples, six samples (Groundnut soup, Light soup, Okro soup, Nkontomre stew and white oil) had only one bacterial species isolated from each of them. Two samples (Salad and Milk) had eight bacterial species isolates from each of them whiles four samples (Fufu, Beans, Kenkey and Tomato stew) had three species isolated from each of them. Three samples (Red pepper, Gari and Plantain) had six bacterial species isolates from each. Four samples (Chicken, Shito, Rice and Yam) contained four bacterial species each. Five food samples (Koko, Khebab, Palm nut soup, Red oil and Akple/Banku) contained two bacterial species each. A sample (Waakye) had five bacteria species whiles Macaroni had the highest number of isolates of sixteen bacteria species isolates (Table 2).

Discussion

The rate of microbial food safety research in the country calls for concern because of the increasing population and the increasing consumer concern in the country. We found that the trend in the publication of microbial food safety articles is abysmal (Table 1). It is possible that some microbial food safety research papers that are not in PubMed might not be included, but it contains most of the published concerning food safety about Ghana. The analysis performed here implies that researchers (both governmental and private-based) must take into consideration the weakness of food safety research in the especially in the Northern regions of Ghana (Fig. 1). This is very important if the problem of foodborne illness is to be reduced holistically through research findings in order to reduce the government's huge expenditure on foodborne illnesses as stated by FAO. The regions in the North are less endowed and constituted with the poor majority [9], and with high incidence of diseases in the country. Poverty is known to have a direct influence on health in the country [10].

Most of the articles were directed to one intervention. Almost less than half of the articles included multiple bacterial species in their researches whiles three did not describe the pathogen type. It may be cost effective to include more bacteria of interest in the food industry considering the time and transportation expenses involved in sample collection. Most of the publications seem to pay more attention on the post-processing microbial activities and street food with less focused on bacteria activities during the processing stages and the primary production stages (farm). The approach toward producing safer food devoid of microorganisms by monitoring from farm to fork has been found to be very effective [4]. Researchers should therefore take into account researching into microorganisms from farm to folk. We consider very useful a

research done to assess the effect of applying Good Management Practices and the Hazard Analysis and Critical Control Points (HACCP) to traditional food processing at a semi-commercial kenkey production plants in Ghana [11], however it did not meet our inclusion factors for this research. We urge researchers to consider researching into more traditional foods with high risk of microbial contamination in order to give directions on how to manage the microbial hazards associated with them. None of the publications included seafood, shellfish and aqua-cultural products in their research. This calls for concern considering the number of people who consume fish and the fact that fish is recognized as the most important source of animal protein in Ghana [12]. The researchers focused more on the isolation of bacteria from Ruminants (beef, mutton and chevon) with little work done on milk products and vegetables. One major group of food that was missing in all the publication was fruits. This, we think, is a high-risk food to consumers because they are eating without heating, but they are generally not handle hygienically. Fruits such as banana, coconuts juice, orange, mango etc could also be analyzed for their microbiological quality. Research into the microbiological quality of street sold bread may provide useful information on safety of bread since it is one of the commonest street foods, but poorly handled especially in the hinterlands.

The impact of the isolation of these microorganisms from the various food samples cannot be appropriately evaluated without testing them against the common antibiotics used in the area. It is very surprising to note that only one of the articles in this work performed an antibiotic susceptibility test on their isolated microorganisms from food. This article reported the level of resistance of their isolates to the commonly used antibiotics [13]. Information about the antimicrobial resistance levels of the isolates from the other 10 articles could have help policy makers and other

researchers in the country as well as the global fight against antimicrobial resistance. There may be financial constraints or inadequate access to resources when factoring in additional parameters in research. Researchers into microbial food safety in Ghana should try as much as possible to consider antibiotic susceptibility testing in their researches whenever they succeed in the isolation and identification of microorganisms from food isolates. It will be of help if the authors of the articles involved in this meta-analysis can perform antibiotic susceptibility test on the isolates, if they have them stored. Few proven mechanisms exist for antimicrobial resistance control, and almost none has been validated in developing-countries settings [14]. According to this research, we recommend that researchers should consider the isolations of the most dominant isolates (Fig. 2) from food so that we can get information about the levels of resistance to antimicrobials of these microorganisms in food in the country. With our findings, we can say that these dominant isolates may pose risks as far as food safety in the country is concerned. Currently, the European Union is waging a serious Antimicrobial Resistance War in their member countries against some of those common microbes that we found in Ghanaian foods such as *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa.* The recent outbreak in Germany is another challenge [6].

An article reported that food handlers constitute a significant risk in the spread of enteric fever in Kumasi [15]. This calls for concern because the situation could be worst in other town where food safety education is generally low. A nation-wide food safety education campaign using the WHO's five basic keys to ensuring safer food can help to update new and old food vendors. The prevalence of these indicator microorganisms in food must really be considered threatening. Food vendors in the country should be obliged by the law to undergo regular examinations before handling

food sold to the general public. All the municipalities and district assemblies in the country should be encouraged to embrace the concept of Food Safety Supervisors who must constantly check the food vendors. For effective and efficient enforcement of food safety and food laws, the government should try to merge the Ghana Standards Board and the Ghana Food and Drugs Board, which seem to have duplicated functions in certain areas of their operations.

In one of the articles, *Shigella* spp. was isolated from an imported chicken (Table 2), but it was absent in the local ones. This revelation must be treated as a matter of urgency considering the fact that the country is a food deficit country and hence import a lot of food. More than 200 known diseases are transmitted through food [3]. This implies that surveillance on food and food products entering the country must be strengthened to prevent or minimize the inflow of possible infections, which may be imported into the country.

All the articles used classical methods in the identifications of bacteria. None of the articles employed the molecular method of bacterial isolation and identification. The use of these techniques can give more precise or accurate results. The methodological quality of the primary study of all the articles met the required standard and most of them were reproducible, but did not cover a wide scope probably due to financial constraints. Six of the articles did not indicate the Total Viable Counts of the isolates in food. The inclusion of the total count or colony forming unit (cfu/ml) can improve the reproducibility of the articles by serving as a guide for future researchers and help to determine the required acceptable colony forming units. Most (9) of the articles did not propose direction for future research (Table 3). Proposed directions for future researches are useful for the identification of needed and promising areas for future research and may discourage duplication of research efforts

[16]. Eight of the articles included some pathogens of interest in the food production sector, but only a few included some bacterial that are very pathogenic (*Campylobacter* spp., *Salmonella* spp., *Bacillus* spp. etc) in the food chain. The isolation of other important foodborne pathogens of interest such as *Listeria monocytogenes*/*Listeria* spp., *Clostridium perfringens*, *Cronobacter* spp, *Escherichia coli O157:H7* were not considered in any of the publications. It is worrying that most of the publications did not identify their isolates to the species level. This does not help to develop specific interventions for the elimination of these microorganisms in the food chain since some species require special treatments in their eradication.

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Appendix

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Figure legends

Figure 1

The map of Ghana showing the current official regions and the percentages of microbial food safety of published articles involved in the meta-analysis. A research sought to investigate problems in both Accra and Kumasi only representing 9%. A research investigated about a problem in Accra, Kumasi and Tamale only representing 9%. The percentages presented on the left show the percentages of the microbial food safety articles involved in the meta-analysis taking into consideration the southern and northern halves of the country while that at the right shows research carried-out generally in the country.

Figure 2

Frequency of the bacterial Isolates found in all the food samples involved in the meta-analysis. A total of 26 food samples with 105 bacterial Isolates were obtained in the 11 articles.

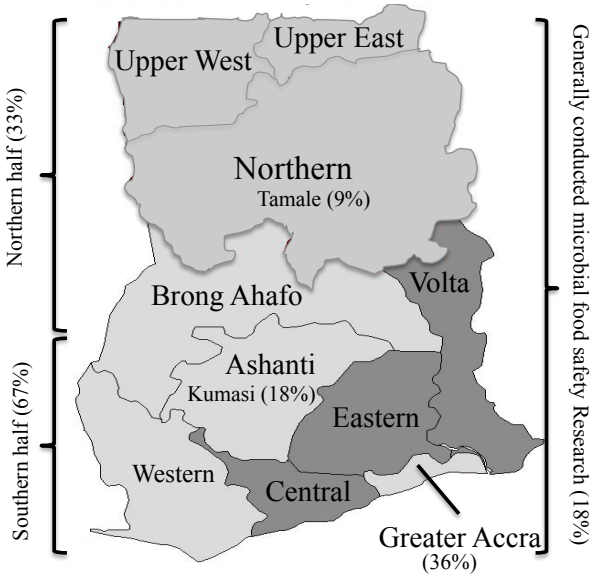

Figure 1.

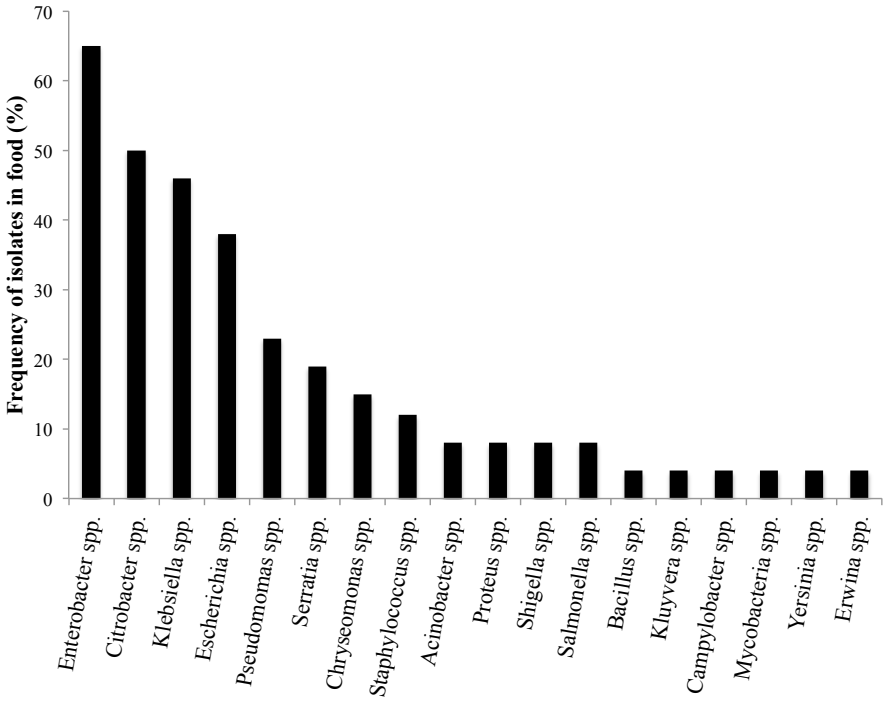

Figure 2.

Table 1. Descriptive statistics of the 11 articles addressing microbial food safety interventions in Ghana published between 1997-2009**.**

	Articles specific to one intervention (n=7)	Articles specific to multiple interventions (n=2)	General articles with a section on interventions (n=2)
Year of publication			
1997	0	0	1
2000	2	0	0
2001	0	1	0
2002	0	1	0
2003	1	0	0
2004	1	0	0
2005	1	0	0
2006	0	0	1
2007	1	0	0
2008	1	0	0
2009	0	0	0
Pathogen type			
Single bacterial species	3	0	0
Multiple bacterial species	2	2	1
Pathogen type not described	2	0	1
Food production sector			
Farm	2	1	1
Processing	1	0	0
Street food	4	1	1
Storage (cold store)	0	1	1
Post-processing	5	2	0
Vendors	3	1	0
Consumers	2	0	0
Commodity group			
Vegetables or crops	0	0	1
Poultry	1	0	1
Swine	1	0	0
Ruminants (beef, mutton, chevon)	3	0	0
Aquaculture, seafood, shellfish	0	0	0
Milk products	1	0	0
Mixed products	1	1	0

Format modified from Sargeant *et al.,* 2006

Table 2. Different foods and the bacteria isolated from them in the 11 analyzed articles.

Food	Bacteria Isolates
Chicken	[10]*Shigella* spp. *(imported)*, [10]*Salmonella* sp., [10]*C. jejuni, and* [10]*E. coli*
Khebab (beef and pork)	[1]*E. coli, and* [1]*Staphylococcus* spp.
Milk	[4]*E. coli*, [4]*Yersinia* spp., [4]*Klebsiella* spp., [4]*Proteus* spp., [4]*Enterobacter* spp., [4]*Staphylococcus* spp., [4]*Bacillus* spp., *and* [4]*Mycobacterium* spp.
Koko	[7]*Chryseomonas luteola,* [11]*Shigella* spp.
Macaroni	[7]*Shigella sonnei,* [7]*P. fluorescente/putida,* [7]*K. pneumoniae,* [7]*E sakazakii,* [7]*E. coli (enteroaggregative diffuse),* [7]*C. freundii,* [7]*S. liquefaciens,* [7]*E. cloacae,* [7]*E. agglomerans,* [7]*E. coli,* [7]*C. diversus/amalonotica,* [7]*Citrobacter* spp., [7]*P. mirabilis,* [7]*Proteus* spp., [7]*E. amnigenus and* [7]*P. capacia.*
Salad	[7]*P. aeruginosa,* [7]*S. liquefaciens,* [7]*E. sakazakii,* [7]*E.cloacae,* [7]*P. fluorescens/putida,* [7]*C. freundii,* [7]*E. coli, and* [7]*C. diversus/amanalotica*
Shito (over cooked stew)	[7]*K. cloacae,* [7]*K. pneumoniae,* [7]*E. cloacae, and* [7]*E. coli*
Tomato stew	[7]*C. freundii,* [7]*E. sakazakii,* [7]*E.coli (enteroaggregative localized)*
Nkontomre strew	[7]*E. cloacae*
Fish	[7]*C. diversus,* [7]*E. coli,* [7]*C. luteola,* [7] *P. fluorescens/putida,* [7]*E. sakazakii,* [7]*C. diversus/amalonatica,* [7]*K. pneumonia*
Palm nut soup	[7]*C. freundii,* [7]*E. cloacae*
Groundnut soup	[7]*C. fruendii*
Light soup (meat)	[7]*Salmonella arizonae*
Okro soup	[7]*E. cloacae*
White oil	[7]*Pseudomonas* spp.
Red oil	[7]*E. hermanii,* [7]*C. freundii*
Red pepper	[7]*K. pneumoniae,* [7]*S. liquefaciens,* [7]*Kluyvera* spp., [7]*E. cloacae,* [7]*E. amnigenus,* [7] *Citrobacter* spp.,
Beans	[7]*C. freundii,* [7]*K . pneumoniae,* [7]*E. cloacae*
Kenkey	[7]*Pseudomonas* spp., [3]*Klebsiella* spp., [3] *Staphylococcus* spp.
Gari	[7]*C. freundii,* [7]*E. cloacae,* [7]*C. luteola,* [7]*Serratia funtida,* [7]*E. aerogenes,* [7] *E. agglomerans*
Rice	[7]*E. coli (enteroaggregative diffuse),* [7]*S. marcescens,* [7]*K. pneumoniae,* [7]*P. fluorescens/putida*
Yam	[7]*C. freundii,* [7]*K. pneumoniae,* [7]*Citrobacter* spp., [7]*C. luteola*
Plantain	[7]*Citrobacter* spp., [7]*K. pneumoniae,* [7]*Acinotobacter* spp., [7]*Klebsiella* spp., [7]*Enterobacter* spp., [7]*C. freundii*
Fufu	[7]*C. diversus,* [7]*E. cloacae,* [7]*E . sakazakii,*
Wakye (rice and beans)	[7]*Enterobacter* spp., [7]*Acinobacter* spp., [7]*Erwina* spp., [7]*E. cloacae,* [7]*K. pneumonia*
Akple/Banku	[7]*E. cloacae,* [7]*K. pneumonia*

The numbers in superscript correspond to the articles meta-analyzed as arranged in the appendix.

Table 3. Criteria for the methodological soundness of the 11 articles involved in the meta-analysis addressing microbial food safety interventions published between 1997-2009

Criteria	Articles specific to one intervention (n=7)	Articles specific to multiple interventions (n=2)	General articles with a section on intervention (n=2)
The article addressed a focused intervention question	6	2	2
The method of locating evidence was described	7	2	2
Explicit criteria was used to select studies	5	2	1
The methodological quality of the primary studies was assessed	7	2	2
Assessment of the study was reproducible	7	2	2
Quantitative summary of the intervention effectiveness among studies was presented	5	2	2
Possible reasons for the difference between studies were presented	5	2	1
The generalizability of the result to target group was discussed	6	2	2
Directions for future research were proposed	1	1	0
Outcomes included the pathogen of interest within the food production sector	6	1	1

Adapted from Sargeant *et al*., 2006

2) First Identification of *Salmonella* Urbana and *Salmonella* Ouakam from Humans in Africa

First Identification of *Salmonella* Urbana and *Salmonella* Ouakam from Humans in Africa

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Abstract

Salmonella is a major and increase cause of morbidity and mortality in both humans and animals worldwide. *Salmonella* surveillance data from Africa countries are very few and the data collected from those surveillance data are only concentrated in major cities. Moreover, continuous surveillance data do not exist on the antimicrobial susceptibility of *Salmonella.* The prevalence and burden of *Salmonella* infections in Africa are normally estimated from just a few surveillance data and does not represent the general situation in Africa. The problem is further compounded by the unavailability of trained personnel, logistics, and inadequate financial support from the governments for scientific research works. We isolated and identified *Salmonella* spp. from possible healthy and sick individuals in Tamale, Ghana with standard microbiological methods. Four different serotypes were identified: *Salmonella enterica* Serotypes Urbana, Ouakam, Stanleyville and Senftenberg. All the isolates were susceptible to all antibiotics tested. This is the first identification of serotypes *S.* Urbana and *S.* Ouakam from humans in Africa.

3) Identification, Molecular Characterization, and Genetic Diversity of *Escherichia coli* Populations from Humans, Animals and Foods from Northern Ghana, West Africa

Identification, Molecular Characterization, and Genetic Diversity of *Escherichia coli* Populations from Humans, Animals and Foods from Northern Ghana, West Africa

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Abstract

Background

Reports from WHO indicate that majority of those who suffer annually from foodborne related diseases are from developing countries, especially from sub-Saharan Africa. However, there is little information in these countries to help curb these mostly preventable foodborne related diseases. Further, antimicrobial resistance is becoming a problem in the region. Here we assess a systematic analysis of *E. coli* in street-food, animals and healthy and ill humans in the Northern region of Ghana.

Methodology

We isolated and identified *E. coli* with PCR from humans, animals and food to determine their phenotypic and genotypic resistance to antibiotics. PCRs were performed using specific primers to amplify the $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$, *qnr* genes in the isolates that were resistant to third generation cephalosporin and reduced suceptibitlity to ciprofloxacin. We digested and extracted plasmids with S1 enzyme and Qiagen Midi-kit and checked for the presence of class one integrons (*intI*) and integron cassettes. We performed multiplex PCR to amplify the Shiga toxin genes, *stx1 and stx2*, and performed a triplex PCR to characterize the isolates into *E. coli* phylogenetic groups. Plasmids were analysed using a PCR based replicon typing. We performed PFGE with *XbaI* enzyme of all the isolates.

Principal Findings

High fecal contamination was found in street food. Antimicrobial resistance levels in all bacteria were lower than in developed countries. Resistance to third generation cephalosporin was only found in the hospital isolates, (BB1094 and BB1095), that were positive for $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$ and class one integrase (*intI*). The BB1095 had a fused conjugative plasmid of 97 kb with three replicons (IncFII, IncFIB and IncY)

which bore an additional *qnrS1* gene but had no gene cassette in its integron while the other has a non-conjugative plasmid with the replicon type (IncU) and an integron bearing a *dfrA1* gene cassette. None of the strains was positive for Shiga toxins. The dominant phylogenetic group from the isolates was group A followed by group B1, but group B2 and D were found in human and animal isolates. PFGE results showed genetic relationship among *E. coli* isolates from humans, animals and food.

Conclusions

There is a flow of *E. coli* in human, animal and food bacteria in the Northern Region of Ghana. Resistance to third generation cephalosporins has emerged in *E. coli* isolates and there is high rate of resistance to common antibiotics especially in human and poultry. Interventions are urgently needed to tackle the high-level fecal contamination of street foods in the Northern Region of Ghana.

“A man has always to be busy with his thoughts if anything is to be accomplished.”
—Antonie van Leeuwenhoek

Discussion

5.0 Discussion

This Thesis is being presented with 3 manuscripts that are in various stages of publications. Most of the key findings have been discussed in each manuscript that constitutes this work. To avoid unnecessary repeats of most of the discussions presented in the publications, I will generally discuss some salient points I raised in the introduction and briefly highlight on some of the important results in the three publications of this Thesis.

5.1 Food safety and foodborne illnesses: Africa versus the rest of the world

One question I repeatedly ask myself is: If the USA, the most technological advanced country in the world, and other developed continents like Europe and Australia are battling with the problem of food safety and foodborne illnesses despite having well-structured food and health systems, **what is happening to the resource-limited or poor countries?** Certainly, the situation in African countries and other developing countries should be overwhelming. The general situation of food safety in most countries seems to be attracting attention day in, day out because of the increasing awareness on food safety issues.

Moreover, the research findings from most of the advanced countries are pointing to the fact that more people are getting sick from foodborne illness and the increased hospitalization days are becoming a serious burden on individuals, the society and the governments (Scallan *et al*., 2011a; OzFoodNet, 2010; Scallan *et al*., 2011b; Pires *et al.*, 2012; EFSA, 2012). However, a significant success chalked in New Zealand from 2007-2010 was that they reduced

campylobacteriosis and salmonellosis in the food chain by 44% and 10% respectively (Table 4).

The important roles played by both national and international bodies to curb the ever-increasing food safety and foodborne illnesses cannot be overemphasized. The WHO in particular, through its affiliate organizations like FAO, Codex Alimentus, WTO and other international bodies like the ISO, IFPRI, OECD, ILSI, IAFP, IPPC, among others have played very important roles in the advancement of food safety worldwide. However, a lot still remains to be done especially in the resource-limited, countries particularly in Africa.

While the international bodies are doing their best to promote food safety issues, resource-limited countries must endeavor to collaborate with them in order to help curb food related issues in their respective countries. Even though most African countries did not put food safety issues high on their national agenda around the 1980s (Käferstein, 2003), they are now realizing the importance of food safety. Some African countries now have food safety policies while others are in the process of enacting food safety laws and regulations for the benefit of their citizens. African governments must also collaborate with the academia and industry to help promote research into food safety and educate the general population on food safety issues (Figure 2).

The AU should focus on more health and food safety programs among its member states just like the EU, rather than the present political bias AU. This will go a long way to help member states to collate and share data on foodborne illnesses and other health related issues in the continent. The AU must also strengthen information technology among its member states to facilitate the easy

transfer of data among countries. As compared to EU, USA and Australia, the AU has no effective and efficient food safety policy let alone reporting foodborne illnesses among member states.

Even if there was a food safety policy and educational programs among members, the populace of member countries will still face the problem of inaccessibility to those policies and educational programs because of inadequate internet access in the continent. The high illiteracy rate as well as the overwhelming number of languages can also be a hindrance to the education and data sharing within and among countries. As a result of ignorance or culture, some popular sayings among some African countries encourage the consumption of unwholesome foods. In Ghana and other African countries, a phrase such as "African germs do not cause any harm or kill" is popular especially among the rural inhabitants. In Equatorial Guinea the popular saying is "An Equatorial Guinean does not die from a disease, but as a result of witchcraft" or "a running stream or river does not kill when one uses water from it". To my surprise also, I heard such phrases in Europe (Spain), "what does not kill you, fattens you" is commonly said when someone picks up and eats a piece of food that has fallen on the ground.

While most Spaniards are more informed about the harmful effects of some microorganisms, most people in Africa have little information on the harmful effects of microorganisms. These phrases, which I think were handed down from generation to generation, must be discouraged if food safety education is to have a positive impact on the people. Some people in Africa still believe that microbial infections are caused as a result of infidelity or punishment from their 'gods'. A

practical approach must be developed to demystify those believes by scientifically based demonstrations without an aggressive attitude towards the beliefs they already hold. One of the critical means of reducing the consumption of unsafe foods or microbial infections in Africa will be the use of the microscopes to demonstrate to the populace and convince them that microorganisms really exist. This may sound unsurmountable but it is should be considered as a key issue.

5.2 Food Safety Versus Food Security in Africa

One of the critical issues in Africa, that I think pushes food safety issues into oblivion is food security. The inability of both colonial and present governments to focus on key food safety issues rather than food security in the past have also affected the evolution of food safety in Africa. Unlike countries in Africa who now struggle to get sanity regulations in the meat industries (meat inspection), the USA for instance started evolving on these important issues in the 1900s. The major issues tackled by colonial governments were food security in order to reduce the hunger situations in the continent and subsequent independent governments also followed in the same direction without paying attention to food safety. The situation is now having a ripple effect on some African economies on the international markets because of their inability to meet food safety standards. The onus is now on African governments to pay equal attention to food safety and food security issues. Just as food insecurity poses humanitarian crisis in Africa, food safety education also deserves attention due to the number of children who die annually from diarrhea diseases in Africa (WHO, 2011).

5.3 Food safety in Ghana

Most street foods in Ghana are not warmed above the constant recommended temperature for storing hot food (63°C or above) after cooking, and cold food (below 5°C) (Mensah *et al.,* 2002). The situation in African countries whereby food is held under ambient temperature coupled with unhygienic practices may present a perfect growth condition to microbes such as *Bacillus cereus, Staphylococcus aureus, E. coli* to multiply rapidly and cause diseases in man. The risk of contamination of food with *S. aureus* is very high due to its presence on the skin, mouth, nose and the frequency of hand contact of food by most people in Africa. The risk of contamination with *Bacillus cereus* is due to the fact that most foods, especially street foods, contain cereals and starchy food as they serve as a suitable growth medium for them. *B. cereus* may also be a risk because of the frequent reheating of food in Africa.

The knowledge of Ghanaians about microorganisms is generally low. In a personal analysis of the programs of over 50 tertiary institutions present in Ghana as of 2012, less than 20% have basic microbiology or related courses as a subject. This influences the population significantly because majority of students who graduate from those institutions may not have basic knowledge in microbiology. If the problem of food safety and other infectious diseases are to be curbed in Ghana and other African countries, the key issue is to make sure that students are taught the basics of microbiology or related subject at least once along the educational ladder. I think this will go a long way to break the notions of some who do not know the destructive effects of microbes.

Another problem in Ghana is the overlapping functions of the authorities (GFDB and GSB) involved in the enforcement or regulation of food laws. The government must set up a commission to synchronize the functions of both authorities in order to assign them a specific role or merge the two authorities together to make them more comprehensive, effective and proactive. Similarly, duplicated functions of authorities were experienced in the Netherlands, but a more efficient and comprehensive authority was formed after a merger to form one single unit of food regulation body.

5.4 Malarial Illnesses Versus Food safety and Foodborne Illnesses in Africa

As indicated in figure 4, deaths related to foodborne illnesses outweigh deaths caused by malaria in Africa according to the brief review I did on this topic in the introduction. The global commitment to the reduction of foodborne illnesses (through the promotion Food Safety) is slower than the global commitment to malaria. Thus, the international communities must be aware of these disparities and make the necessary amendments to focus their priority on food safety issues to forestall the deaths associated with foodborne illnesses. Unlike malaria, which has seasonal peak periods, geographically based and can be influenced by the economic status of the victims, the danger of eating unsafe food is a daily affair that cuts across all the social classes and does not respect geographical and international boundaries.

5.5 A brief discussion on *Salmonella* and *E. coli* Research in Ghana and Africa.

In the brief reviews I did in the introduction, I realized that there are some countries that did not have any publication on *Salmonella* and *E. coli.* Generally microbiological research in Africa is very low as compared to the rest of the continents (Figure 7). Once again, African countries must embrace microbiolgical research in their respective countries and support infectious disease surveillance programs in order to get first hand information about diseases and make the appropriate interventions necessary to ameliorate the situation.

The underlying factor of the high incidence of Salmonellosis and *E. coli* infections and most infectious and communicable diseases in Africa could be traced to unhygienic practices (Figure 5). I observed that unlike the Black Death (caused by *Yersinia pestis*), which befell Europe since the 1348 AD and killed one-third or half of the whole population by 1750 (Haensch *et al.,* 2010), African countries have never been hit by such a severe epidemic that cut across the whole continent with such casualties. Unlike Africa, the Black Death revolutionized the hygienic practices in Europe since the population realized that the key to this fatal disease was hygienic practices, but it was not a punishment from God like their African counterparts still believe now. Up to now most Africans do not believe that the key to most of the diseases that befall the continent is caused by unhygienic practices.

A practical example of the beneficial effects of hygiene was the reduction of 50% of typhoid cases after the improvement in sanitation in Great Britain in 1875 Swith (1955, cited by Yoshikawa, 1980). I believe that for a greater improvement

to be made in the reduction of Salmonellosis and other infectious and communicable diseases, African countries must enforce strict sanitation laws and educate the population to convince it on the existence of microorganisms, the way they are transmitted, and how to prevent them. Even though the sources and causes of salmonellosis and *E. coli* infections are widely known among the population in developed countries for the past decades or century, a majority of the population in Africa and other developing countries are deficient of this information. A typical example is the continuous use of rodent feces popularly called grasscutter (*Thryonomys swinderianus*) as a flavoring agent in some communities in Ghana even though such animal could be a reservoir of *Salmonella* and other infectious diseases. Microbial risk assessment research could be done on the feces of these animals to check for the risk of transmission of infectious microorganisms in communities who still cook with the feces of grasscutter. Behavioral and cultural changes may also be a key factor in the reduction of infectious diseases in Africa because of some religious and cultural believes (Opare *et al.* 2000).

There are probably a few or no outbreak reports on *Salmonella* or *E. coli* in Ghana because (i) there are few hospitals or health posts in the regions (ii) there are no or very few diagnostic laboratories in some parts of the regions (iii) the population are ignorant about the symptoms of salmonellosis or *E. coli* infections; some of the populations or healthcare assistance mistake symptoms for other diseases such as malaria and (iv) the apathy to seek for medical attention at the health posts or hospitals.

5.6 Antimicrobial resistance in Ghana and Africa

Generally, there are only a few publications on antimicrobial resistance research in Ghana and the African continent as a whole. The antimicrobial resistance burden is imagined to be high, but there are only a few studies to verify the hypothesis (Okeke *et al.,* 2007). The situation is a serious one because in recent times, most countries, especially in Europe and the US are waging a serious war on antimicrobial resistance due to the increasing number of resistance isolates of bacteria to many classes of antimicrobials. Furthermore, the increasing numbers of resistant strains to antimicrobials present a serious situation whereby very few antimicrobials exist for therapeutic use in hospitals and the development of new ones is very slow. Even where a new antimicrobial is developed, there is fear that it will soon be ineffective against bacteria.

What needs to be done in Ghana and other African countries to combat the increasing resistance to antimicrobials is (i) a national policy on antimicrobial therapy (ii) strict efforcemnet of the policy (iii) effective antibiotic stewardship programs (iv) continuous antibiotic monitoring and surveillance (v) and a holistic approach to antibiotic education. One main situation that still worries me and continues to be on top of my research priorities in Ghana is the efficacy of antibiotics sold in the country. Even though I do not know of the exact figure, most of the pharmacies, especially in the rural areas, popularly called 'drugstores' do not have the right environment for storing antibiotics. Most of them leave their antibiotics to the mercy of the weather. Even where the environmental condition is quite conducive, there is still the issue of the authenticity and efficacy of antibiotics due to the large chunk of fake antibiotics in the market.

Nonetheless, one of the problems that still remain to be tackled in the fight against antibiotic resistance in Africa is the use of antibiotics in animal production for growth promotion. Unlike the EU, which has banned the use of antibiotics in growth promotion in animal production since 2006, most African governments do not have control over the use of antibiotics in animal production. This may result in widespread use of important clinical antibiotics in animal production. What may worsen the situation is that some of those animals treated with medically important antibiotics are left to roam freely (figure 5) among the human population and areas where children play. Contamination of the area with their feces may lead to a possible transmission of resistant bacteria to children who may spread them in their homes. Another repercussion of the uncontrolled use of antibiotics in Africa for growth promotion or therapeutic purpose is the fact that some communities rely solely on nearby streams or rivers as their sources of drinking water and for other domestic chores in the house. Feces of animals treated with medically important antibiotics may contain resistant strains that could get into the river or streams through surface runoff due to excess water from rain. What is more disturbing is that some of the animals also drink from the same streams or rivers used by the people in some communities in Africa.

Strictly speaking, the majority of a population who do not know the existence of microorganisms is less likely to understand the mode and molecular bases of the spread of antimicrobial resistance organisms. The most important way of curbing drug resistance in Africa will largely hinge on the education of the populace about the existence and mode of mechanisms of resistance to drugs or antibiotics.

5.7 Emerging and increasing resistance to antimicrobial in the Northern Region

Comparing this work to a similar work done in the area in 2008 (Djie-Maletz *et al.,* 2008), there is an increasing resistance to antimicrobials in the area but little is known about the genetic basis in Ghana. In 2008, resistance to third generation cephalosporins was not recorded in the Northern Region of Ghana, but our present study shows that this resistance has emerged in *E. coli* in the area.

Furthermore, these strains were ESBL producing strains that were resistant to multiple antibiotics. The strains produced CTX-M-15 enzymes that are now known as pandemic strains displaying multiple drug resistance to antibiotics (Naseer *et al.,* 2011). Some of the strains that emerged from the recent outbreak in Europe that killed over 50 people were reported to possess the CTX-M-15 enzyme (Januszkiewicz *et al.,* 2012). The detection of these types of strains in this work is very important since it will serve as an important baseline for authorities to know that, the strains causing outbreaks have already emerged in the area. The strains that produced the CTX-M-15 enzymes were also found to produce other types of resistance enzymes such as the TEM-1 beta lactamase enzyme, plasmid-mediated quilonone gene, *qnr,* and an integron encoded gene, *dfrA*1, known to produce resistance to trimethoprim.

5.8 The importance of this work to Animal and Public Health in Ghana

The results of this Ph.D. Thesis serve as baseline studies, especially for other molecular characterization studies of antimicrobial resistance in the Northern Region and Ghana as a whole. While the world has become more technologically advanced and most public and animal health officials in other countries are employing molecularly based diagnostic techniques, Ghana and other African countries are yet to catch up with the rest of the world. We used PFGE to characterize all the *E. coli* strains we isolated from humans, animals and street foods. The results will help public health officials in the Region to know the genetic relationships among the different strains of *E. coli* from different sources to establish the sources of contamination of street foods, thus whether the source of contaminations of street foods are from the food vendors or from food animals to street foods because vendors purchase these animals and their products to prepare the street foods. And more importantly, to know if patients are carrying the same strains of *E. coli* isolated from street foods and animals. This will assist the public health officials in their duty to prevent a possible outbreak of foodborne illnesses, not only as a result of *E. coli* but other pathogenic zoonotic organisms like *Salmonella*, *Campylobacter and E. coli* O157 in the Region.

Moreover, the results will help Veterinary officials in the Region and the whole of Ghana to know the level of antimicrobial resistance in farm animals and take necessary precautionary measures to help in the fight against antimicrobial resistance in both the animal population and humans.

Finally, this Ph.D. Thesis could not have been completed without any hindrances throughout the 4-year period of its preparation. One major difficulty

we faced was improper record keeping on the patients from which we took our hospital samples. This prevented us from linking some of the results to age, gender, and disease symptoms. In the meta-analysis of microbial food safety publications, we only had a few articles published on PubMed from Ghana that met our inclusion criteria. We suggest that more molecular and food safety studies should be carried out in the Northern Region and other parts of Ghana in order to get more representative results to estimate the true burden of antimicrobial resistance and foodborne diseases in Ghana.

“All is well that ends well.”
—William Shakespeare

Conclusions

Conclusions/Conclusiones

First: The most frequently isolated bacteria from foods from Ghanaian publications were *Enterobacter spp.*, *Citrobacter spp., Klebsiella spp.* y *Escherichia spp.,* representing 65%, 50%, 46% and 38% respectively.

Primera: Las bacterias más frequente aisladas de los alimentos en las publicaciones en Ghana fueron *Enterobacter spp.*, *Citrobacter spp., Klebsiella spp.* y *Escherichia spp.,* con porcentaje 65%, 50%, 46% y 38% respectivamente.

Second: We observed that the annual death rate of foodborne illnesses and related cases in Africa are far higher than death from malaria but still there are more interventions to control the latter.

Segunda: Hemos observado que el numero de muertes anuales causadas por enfermedades transmitidas por alimentos y afines en África es más alto que el numero de muertos por malaria pero hay más intervenciones para controlar la segunda.

Third: For the first time we report the presence of *S.* Urbana and *S.* Ouakam in human in Africa and *S*. Stanleyville and *S.* Seftenberg from the Northern Region of Ghana.

Tercera: Hemos descrito por primera vez la presencia de *S*. Stanleyville y *S.* Seftenberg en la Región Norte de Ghana y *S.* Urbana y *S.* Ouakam procedentes de humanos en África.

Fourth: Thirty percent (30%) of street foods (Waakye, Jollof, Macaroni) in the Northern region of Ghana were fecally contaminated.

Cuarta: El 30% de los alimentos procedentes de la venta ambulante en las calles de la region Norte de Ghana presentaron contaminación fecal.

Fifth: There was high rate of resistance of *E. coli* to commonly used antibiotics isolated from humans, animals and street foods from the Northern Region of Ghana.

Quinta: Se obsevó un alto nivel de resistencia en aislados de *E. coli* de origen humano, animal y alimentario de venta ambulante a los antibióticos de uso común.

Sixth: For the first time, we have identified and characterized the resistance of *E. coli* to third generation cephalosporin in the Northern Region of Ghana.

Sexta: Hemos identificado y caraterizado por primera vez la resistencia de *E coli* a las cefalosporinas de tercera generación en la región Norte de Ghana.

"Every thought is a seed. If you plant crab apples, don't count on harvesting Golden Delicious."
—Bill Meyer

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